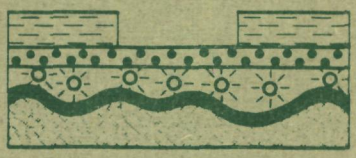
the measurement and the regeneration of the water vapor loss of human skin

meting en herstel van de water-barrière van de huid van de mens



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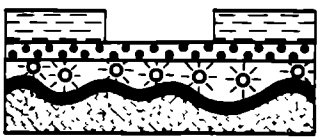

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the measurement and the regeneration of the water vapor loss of human skin

PROEFSCHRIFT

ter verkrijging van de graad van doctor
in de wiskunde en natuurwetenschappen
aan de katholieke universiteit te Nijmegen,
op gezag van de rector magnificus
mr. S.F.L. baron van Wijnbergen,
hoogleraar in de faculteiten der rechtsgeleerdheid
en der sociale wetenschappen,
volgens besluit van de senaat
in het openbaar te verdedigen
op vrijdag 25 april 1969,
des namiddags te 2 uur precies

door

David Spruit

geboren te Zetten

1969

offsetdrukkerij minOprint
Nijmegen

Jahweh, mijn hart is niet trots,
niet hovaardig mijn ogen;
ik houd mij niet op met geweldige plannen,
met dingen, die te hoog voor mij zijn.

Neen, ik voel mij zo klein,
en beeld mij niets in;
zoals de zuigeling aan de borst van zijn moeder
ben ik een kindje voor U.

Israël, stel uw hoop op Jahweh,
van nu af tot in eeuwigheid.

psalm van David

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CONTENTS

CHAPTER I

INTRODUCTION

Summary

In order to improve our understanding of the phenomena related to the water vapor loss of skin, several factors are considered which have already been established as being of importance in the behavior of synthetic and biologic membranes. According to the laws of diffusion and permeation the transport of material is governed by a driving force, the concentration or pressure gradient through the membrane, and a characteristic of the membrane, the permeation coefficient. The temperature, the absorption of water (swelling), and the structural characteristics of the membrane influence the permeation.

I.1 GENERAL REMARKS

"Die Hydrodiffusion durch Membranen dürfte billig "nicht bloss als einer der Elementarfactoren des organischen "Lebens sondern auch als eine an sich höchst interessanter phy- "sikalischer Vorgang weit mehr Aufmerksamkeit der Physiker in "Anspruch nehmen als ihr bisher zu Theil geworden ist."
This is the first sentence of Adolf FICK's article: "Ueber Diffu- "sion" in Poggendorff's Annalen der Physik und Chemie, 94: 59-86 (1855). FICK knew very well that a lot of work had already been done; he himself refers to the "Porentheorie" developed by BRUCKE, theories of POISSON and of BECQUEREL, experimental work of JOLLY, of LUDWIG, and of CLOETTA. At that time, he certainly was not the only one working in this field. Yet,

according to FICK: "Vielleicht ist der Grund dieser spärlichen "Bearbeitung zum Theil in der grossen Schwierigkeit zu suchen, " auf diesem Felde genaue quantitative Versuche anzustellen."

Nowadays there is "sufficient indication that water "structure is very probably involved in biological processes "and that it is worthwhile to concentrate experimental efforts on "the study of these effects" (BERENDSEN, 1966). Conferences were held in New York, 1964, on "Forms of water in biologic "systems", and, 1964, on "Cryobiology", and at Stowe-Vermont, 1965, a.o. "Water" and "Membrane transport" in collagenous tissue. A century has elapsed since FICK's statement was made. Many aspects of hydrodiffusion through membranes are not yet understood, but its importance is widely recognized. In the meantime the methods of measurement have been improved very much as may be evident from the 1963 International Symposium on Humidity and Moisture at Washington (A. WEXLER: Humidity and Moisture. Measurement and Control in Science and Industry. Vol. I-IV. Reinhold Publ. Co., New York, 1965).

Many methods have been described by which the water vapor loss of skin can be determined. A review has been given by HEERD (1959), who classified most methods in his thesis. Moreover, he added his own new method. During the following years, further, new methods were described in scientific journals. The dermatological clinic and the department of medical physics of Nijmegen University contributed to this series with an electrolytic water analyzer method (van GASSELT & VIERHOUT, 1963). In 1966, SPRUIT & MALTEN described an improvement of this method. The electrolytic water analyzer method turned out to be very sensitive, though a considerable time is required for a measurement to be completed, and it necessitates passing a real dry atmosphere over the skin. Two years

afterwards another method has been proposed by SPRUIT, in 1967. This method - to be described in Chapter II of this thesis - allows ambient humid air to pass over the skin and an immediate recording of the skin's water vapor loss to be made. Both methods have been applied in measurements described in this thesis (see Chapter III).

The hydrodiffusion through skin is well known as the water vapor loss of skin or its so-called "insensible perspiration". It is a vital element in life, as the skin protects the body from desiccation. Extreme, dramatic consequences of a failure of the protective function in old age have been described by LEERING (1966; 1968). Apart from such extreme consequences, the insensible perspiration of normal and pathologic skin is an important physical characteristic of skin; the value of the insensible perspiration will inform the investigator about the quality of the skin in respect of its protective functioning. The estimation of the insensible perspiration quantitates a function of the skin and hence can be a real contribution to the dermatologist's visual observation of pathological skin, adding a quantitative datum to his qualitative impressions. It provides supplementary information about the regeneration of injured skin (Section IV.3), and it allows the regeneration of skin to be followed even when its appearance has already become "normal" (Chapter IV, formation of the so-called "final barrier").

I.2 PERMEATION OF WATER THROUGH A SYNTHETIC MEMBRANE

According to MOLL (1964) and many others, BARRER's theoretical considerations of permeation through polymer membranes (1958) have proved to be the best ones so far available. In a simplified way, BARRER's theory is followed in the next pages.

Material (e.g. water) will only pass an interface, when a concentration difference - $(C_1 - C_2)$ - exists between the two sides of the interface. This concentration difference is the driving force of the transport through the interface. The amount of material (M), transported through an interface per unit of area (A) and per unit of time (t), is directly proportional to this driving force and follows the relation:

$$M / A\,t = D\,(C_1 - C_2) \qquad (1)$$

The proportionality factor (D) is called the diffusion coefficient or diffusion constant.

The amount of material transported per unit of area and per unit of time may be represented as g cm^{-2} sec^{-1}, though the water vapor loss through the skin is, in practice, usually measured in mg cm^{-2} h^{-1}; the latter unit will be used in this thesis. According to formula (1) the diffusion coefficient (D) has the dimension of cm sec^{-1}, as the concentration is measured in g cm^{-3}. In evaporation studies of water through monomolecular layers the same formula is used though instead of the diffusion coefficient the reciprocal dimension is preferred;

$$r = 1 / D \qquad (2)$$

r being the resistance of the layer against transport, and is evaluated in sec cm^{-1}. In this case formula (1) is read:

$$r = \frac{(C_1 - C_2)}{M / A\,t} \qquad (3)$$

When material has to pass several layers having different resistances against transport of the material, formula (3) is useful.

The diffusion coefficient is not only described as in formu-

la (1); it can be used as a specific material constant by calculating the diffusion coefficient per unit of thickness of the material through which the transport occurs. This specific material diffusion coefficient (D_s) is, therefore, read in $cm^2\ sec^{-1}$ and formula (1) is transferred into:

$$M / A\ t = D_s\ (C_1' - C_2') / d \qquad (4)$$

D_s is encountered in studies concerning the diffusion of material through synthetic membranes of known thickness. In these studies another factor is important too. The way in which the transported material is distributed between the membrane material and the outside milieu depends on the partition coefficient of the transported material between these phases. The effective concentration, relevant to the "driving force" of formula (4), the concentration difference $(C_1' - C_2')$, is dependent on the solubility coefficient according to:

$$S = C' / C \qquad (5)$$

C' being the concentration of the material in the membrane when the concentration in equilibrium at the outside of the membrane is C, which concentration is easily determined in permeability experiments.

Accordingly formula (4) can also be read as:

$$M / A\ t = D_s\ S\ (C_1 - C_2) / d \qquad (6)$$

which is more easy to handle experimentally. The product of both constants D_s and S is known as the permeation coefficient or permeation constant (P):

$$P = D_s\ S \qquad (7)$$

and as S reads in $cm^3\ cm^{-3}$, the dimension of P and D are the

same, $cm^2\ sec^{-1}$. Consequently formula (6) can be read:

$$M / A\ t = P (C_1 - C_2) / d \qquad (8)$$

the permeation coefficient being a specific material constant.

In many experiments using synthetic and biologic membranes the permeation of gases or vapors is investigated and the concentration of the gas is not estimated as such but as a vapor pressure (p), which is related to its concentration at the surface of the membrane according to HENRY's law:

$$S_g = C' / p \qquad (9)$$

Consequently the S_g is read in $g\ cm^{-3}\ (cm\ Hg)^{-1}$. Similarly to the above formulae (6), (7), and (8), the following formula can be applied in experiments dealing with the transport of gas or vapor through a membrane:

$$M / A\ t = P_g\ (p_1 - p_2) / d \qquad (10)$$

in which formula the dimensions of P_g deviate from the dimensions of the P of formula (8). Whether P_g or P is used, is apparent from the unit applied; either $g\ cm\ cm^{-2}\ sec^{-1}\ (cm\ Hg)^{-1}$ (JOUWERSMA, 1959) or $cm^2\ sec^{-1}$ (MOLL, 1964) is applied. As a simplification the index "g" will be omitted in the following text, whether P_g or P is meant.

The above mentioned considerations are simplified ones. In reality HENRY's law is only valid when the solution is very dilute. In the case of water, S is increased at high concentrations of water when clusters of water molecules are formed (MOLL, 1964). Accordingly, when water is the material to be transported through a membrane, the use of these laws appears to be justified in the case of a hydrophobic membrane, as has been proven experimentally (MYERS et al., 1961; MOLL, 1964). It may not be justified in the case of a hydrophylic membrane,

as biological membranes are. In measurements at synthetic keratin membranes, CASSIE (1945, 1946) found deviations of the "constant" permeation coefficient only when the water content of the keratin membrane was increased above 16 %. The water content of the stratum corneum (horny layer) of normal healthy skin has been estimated as 2 - 10 % (SZAKALL, 1957; MIDDLETON, 1968). It might, therefore, be supposed that HENRY's law will be valid in normal circumstances and that the permeation coefficient of the stratum corneum will, in fact, be constant. However, this supposition has proved not to be justified (SPRUIT & MALTEN, 1969[b]; this dissertation, chapter IV).

The permeation coefficient and the diffusion coefficient are temperature dependent and so is the solubility. Accordingly it follows from formulae (7) and (9):

$$P(T) = D(T)\ C(T) / p(T) \qquad (11)$$

T representing the absolute temperature. The dependency of the diffusion constant on temperature, D(T), is represented by the formula:

$$D(T) = D_o\ e^{-E_D/RT} \qquad (12)$$

(see BROWN & TUWINER, 1962, pp. 226-229), D_o representing a temperature-independent constant, E_D the activation energy for the diffusion, and R the gas constant. It is further assumed that HENRY's law still holds when the water vapor has reached its maximum, saturation value. Consequently:

$$C(T) / p(T) = C_m(T) / p_m(T) \qquad (13)$$

According to the equation of CLAPEYRON:

$$p_m(T) = p_o\ e^{-W/RT} \qquad (14)$$

W representing the heat of evaporation, and the similar equation, derived from elementary thermodynamics:

$$C_m(T) = C_o \; e^{-L/RT} \tag{15}$$

L representing the heat of solution, the dependency of C and p on temperature is described by:

$$C(T) / p(T) = C_o / p_o \; e^{(-L + W) / RT} \tag{16}$$

As a consequence from formulae (11), (12), and (16):

$$P(T) = \frac{D_o \; C_o}{p_o} \; e^{(-E_D - L + W) / RT} \tag{17}$$

As also may be written:

$$P(T) = P_o \; e^{-E_P / RT} \tag{18}$$

E_P representing the activation energy for the permeation, it follows that:

$$E_P = (E_D - W + L) \tag{19}$$

In the permeation of water through a keratin membrane L has been estimated 3.5 Kcal/mole (CASSIE, 1946) and W of water is 10.5 Kcal/mole, so that:

$$E_P = (E_D - 7) \quad \text{Kcal/mole} \tag{20}$$

The energy for activation of permeation of water through keratin (and probably also through the stratum corneum of human skin) is about 7 Kcal/mole less than the energy for activation of diffusion of water through keratin. For the sake of comparison it can be mentioned that the difference between the activation energies for permeation and diffusion of water vapor

through poly-ethylene has been estimated 5.5 Kcal/mole (KLUTE & FRANKLIN, 1958).

The permeation of water through human skin is governed by the so-called barrier, located in the outermost anatomical layer, the stratum corneum or horny layer, about 0.03 mm thick (BLANK, 1952 and 1956). The dependency of the permeation of water through human skin on temperature is determined in vitro by the "energy for activation of the permeation" of water through the stratum corneum. This latter value has been measured as about 15 Kcal/mole for the permeation of water through keratin (CASSIE, 1945, 1946), through hair (HOLMES, 1964), and through human stratum corneum (SCHEUPLEIN, 1966; SPRUIT, 1966). The dependency of the permeation of water through human skin on temperature in vivo, however, may be a more complicated figure than the in vitro permeation is (SPRUIT, 1966; SPRUIT & HERWEYER, 1967; THIELE & van SENDEN, 1966). It has been supposed that hydration phenomena are involved.

It is obvious that structural characteristics influence the permeability of the membrane. As an example the permeabilities of high-density and low-density poly-ethylene differ very much. These polyethylenes are only distinguished by their contents of impermeable crystalline phase (with low density) and permeable amorphous phase, resulting from different pressures exerted during the preparation of the polyethylene. An easily permeable high-density polyethylene with E_P = 8.4 Kcal/mole and $P = 4.4 \times 10^{-8}$ g cm cm^{-2} sec^{-1} $(cm\ Hg)^{-1}$ and a less permeable low-density polyethylene with E_P = 14.5 Kcal/mole and $P = 2.0 \times 10^{-8}$ g cm cm^{-2} sec^{-1} $(cm\ Hg)^{-1}$ illustrate the relative deviations caused by different contents of "crystallinity" (KLUTE & FRANKLIN, 1958). The activation energy of this less permeable low-density polyethylene is about the same as the activation energy of the keratin membrane and the stratum

corneum (15 Kcal/mole). On the contrary, the low activation energy for permeation of water through the other polyethylene (8.4 Kcal/mole) shows that the diffusion of water through this membrane can proceed almost as easily as through water itself (activation energy for the self-diffusion of water: 5 Kcal/mole (BROWN & TUWINER, 1962, p. 46)).

The water permeability of hydrophobic membranes (e.g. polyethylene) is influenced slightly by the weak interaction between water and the membrane material (MYERS et al., 1961). The influence is more considerable in hydrophilic membrane materials. A decrease of the water content of a collagen membrane from 50 to 5 % was found to be associated with an increase of the activation energy for the permeation of water from 11 to 14 Kcal/mole (KANAGY, 1950). When the water content of swollen keratin membranes with 16 % water (equilibrated with 80 % RH (relative humidity of the surrounding atmosphere)) was decreased to 8 % water (equilibrated with 30 % RH), the activation energy was also increased by 3 Kcal/mole (KING, 1945). The following formula has been proposed, as it can be derived from theoretical considerations of multimolecular adsorption and corresponds with experimental results of adsorption of water at keratin (CASSIE, 1945, 1946; KING, 1945; HILL, 1946):

$$P = F\ e^{-E/RT}\ (B - 0.95\ X)^{-2}\ \ d(A - X)/dA \qquad (21)$$

In this formula F = a constant; $e^{-E/RT}$ = the factors governing the dependency of the permeability on the temperature (see formula (18)); A = the water content of the keratin in mole/100 g keratin; B = the number of moles water per 100 g keratin which may maximally be occupied by adsorbed water at localized sites of the keratin boundary; X = the number of moles water per 100 g keratin which are occupied by adsorbed water at localized sites at the keratin (first layer of adsorbed water). As a result

(A - X) is the number of moles of water per 100 g keratin which have been adsorbed on top of the X moles of water in the first layer of adsorbed water (thus forming second and higher layers) and are relatively mobile. The (B - X) represent the number of moles of unoccupied sites at the keratin boundary. It is assumed that the diffusing water molecules are not only deactivated by encounters with the (B - X) vacant sites but also by jumps between the X occupied sites; because of this effect the factor 0.95 was introduced in the permeability of water through horn keratin.

When the water concentration around a hydrophilic membrane is increased, the membrane swells gradually until a steady state has been reached. A change in the concentration of the permeating water is not instantly and completely reflected as a corresponding change of the rate of diffusion or permeation. Because of hysteresis of the absorption and swelling of the membrane, the ultimate permeation rate depends on whether the water content of the membrane has been obtained by drying a wet membrane or by moistening a dry membrane. KING (1945) observed that the water permeation through a 0.05 mm thick keratin membrane had the same hysteresis as the water absorption of the keratin. The value of the water permeability is, therefore, dependent on the "history" of the membrane.

The self-diffusion coefficient of water is dependent on the concentration of dissolved materials, especially ions. The self-diffusion coefficient of pure water is about 20 % higher than the diffusion coefficient of water in a 1 M salt solution (JONES et al., 1965). The influence of the ions upon the water structure is responsible for this deviation (FENICHEL & HOROWITZ, 1965). As a consequence the permeability coefficient of membranes has to be dependent on the concentration of soluble substances and change with varying concentration of these substances. The permeability of colloidal membranes will be influenced si-

milarly by the ion concentration.

CHAPTER II

MEASUREMENT

Summary

A short review of the available measuring methods is presented, and the reasons are discussed for the choice of the thermal conductivity cell as a sensitive measuring element in the determination of the skin's water vapor loss in environmental humid air. A commercially available GOW MAC thermal conductivity micro cell has been used as a humidity sensor; the apparatus and the techniques used are described in detail; its sensitivity allows the measurement of the water vapor loss of only one cm^2 human forearm skin; the sensitivity is very similar. whether dried or humid air is used. Several calibration methods, both direct and indirect. revealed a sensitivity of about 0.7 mV $(mg\ water)^{-1}$ $(l\ air)^{+1}$. Among these calibration methods a new, simple direct method has been applied. The specificity has been investigated by direct comparison with a specific electrolytic technique, and was found to be adequate. A measurement can be completed within a few minutes when ambient humid air is passed over the skin.

II.1. PRINCIPLES OF SOME METHODS OF MEASUREMENT

As long ago as 1614, it has been shown that a human being

Most of the material of this chapter has been published in the Journal of Applied Physiology 23(6): 994-997 (1967). Discussion with and help from the cooperators of the NV BECKER, DELFT are gratefully acknowledged.

loses weighable amounts of water (SANCTORIUS). This gravimetric method of measurement is still in use (ZÖLLNER et al., 1955; BREBNER et al., 1956; MALI, 1956; ZOON & MALI, 1957; WEBB et al., 1957). The total loss of water is corrected for the water evaporated from the lungs. The technique of the measurement has, of course, been improved very much. This method gives information about the complete "organ" of the human skin; it does not invite application in a dermatological clinic, where the interest lies in local deviations of small areas of the skin.

The investigation of the water loss of limited areas of the skin has always attracted investigators. Methods in which dried air was passed over a limited skin area (e.g. the forearm) were developed (SEGUIN & LAVOISIER, 1789; BARRATT, 1897; SCHWENKENBECHER, 1904; MOOG, 1923; EIMER, 1927; NEUMANN et al., 1941; BURCH & SODEMAN, 1943). ROTHMAN (1921) succeeded in measuring the water vapor loss of only 20 cm^2 forearm skin. No improvement in sensitivity was reported for some decades.

The earlier methods of weighing absorbed water vapor were gradually abandoned when limited areas of skin were involved in the investigation. Absorption of water vapor, followed by another, more sensitive physical measurement became the vogue. For example, the electrical conductance of a lithium chloride cell or a DUNMORE (1938) element was applied and improved by BETZ (1955). Another example: the electrolysis of absorbed water, applied by van GASSELT & VIERHOUT (1963), was improved by SPRUIT & MALTEN (1966). This last method of measurement is the most sensitive one yet available; an area of only 0.2 cm^2 forearm skin is sufficiently large to permit a quantitative determination and recording of the insensible perspiration. Even more sensitive instruments may soon be developed as a result of the development of new sensing elements, e.g. the

radio frequency quartz crystal coated with a hygroscopic material is already ten times more sensitive than the electrolytic water analyzer (KING, 1965).

Most of the methods can only be applied if dried air is conducted over the skin, though it has often been considered a disadvantage that the skin is exposed to dry air instead of the normal environmental humid air. The permeability of the skin depends on the water content of the stratum corneum (chapter III). The water content of the horny layer of the skin alters when the water content of the atmosphere changes. Therefore many investigators prefer to study the water vapor loss of the skin when exposed to air of a fixed humidity, which can be obtained by bubbling the air through a saturated NaCl solution before it reaches the skin (McDOWELL et al., 1954). Other investigators even want to avoid a flow of air along the skin surface, and record the increasing humidity inside a cup placed upon the skin (HEERD, 1959; THIELE & SCHUTTER, 1962). Ideally, the skin should be investigated under unaltered atmospheric conditions, so that the skin does not need time to acclimatize to a changed environment. In some methods of measurement this principle is realized. Environmental humid air is conducted over the skin and hygrometers are mounted in the air both before and after it has passed the skin. Only large areas of skin have been used in these investigations; the sensitivity of the hygrometer is critical. By measuring the absorption of infrared radiation, the investigated area of the skin can be limited to 20 cm^2 (PALMES, 1948; improved by ALBERT & PALMES, 1951; see also JOHNS, 1965). The measurement of the thermal conductivity of the air has been shown to be an improvement, allowing the measurement of the insensible perspiration of 1 cm^2 forearm skin (SPRUIT, 1967; this chapter). In medical investigations such small areas are a necessity, as the deviating skin is often very limited in its extent. Measurements by this last method may

therefore yield medically interesting results.

In the study of sweat secretion, there are special requirements. The amount of the evaporated water is at least ten times the amount of the water vapor lost by insensible perspiration, so that the method need not be so sensitive. However, the water vapor loss has to be recorded very rapidly. For this reason hygroscopic sensors, measuring electrical resistance, are often applied (DARROW, 1934; ROY, 1960; ROSENBERG et al., 1962). The lithium chloride cell or DUNMORE element (DUNMORE, 1938; BULLARD, 1964; ROVENSKY & SAXL, 1964) has also been used to explore the subclothing micro-environment of clothed human subjects (KENNEDY, 1965) and the relative humidity as near the leaf surfaces of plants as 0.75 mm. These elements are commercially available and their characteristics have been well studied (HANDEGORD et al., 1965); an accuracy of about 0.1 % RH is reached in some of them. The same accuracy has been claimed for the microwave refractometer in the measurement of the moisture boundary layer near plant leaves and human skin (GATES, 1965).

Because the thermal conductivity cell was used in this study for the measurement of the water vapor loss in environmental humid air, this particular sensor will be considered in greater detail.

The thermal conductivity cell is a detector of a change in the composition of a gas, whatsoever the change is. It was introduced by SHAKESPEAR in 1916 and called a "katharometer" (1921). Originally, it was applied in the detection of leakages in Zeppelin air balloons and afterwards found wide application in gas analysis and especially in gas chromatography, where it is used as a sensitive, non-destructive detector (LAWSON & MILLER, 1966; VISSER, 1957; LEYTEN, 1967). Its application as a hygrometer was already suggested by SHAKESPEAR

in 1921; however, in practice its use remained very limited (CHERRY, 1965). Yet, as a hygrometer, it has been applied as the sensing element in the recording of sweat secretion by ADAMS et al. (1963). Its usefulness in the measurement of the insensible perspiration of human skin may be predicted from this report.

In principle, the method of measurement of the water vapor loss from the skin is based on a comparison of the water vapor concentrations of the air before and after it has passed over a fixed area of skin. The sensing element is a thermal conductivity cell; this comprises two compartments. The air passes through the first compartment before it reaches the skin. After the air has been humidified by passing over the skin, the air is led through the second compartment of the cell. A thermistor is mounted in each compartment; these are incorporated into the arms of a WHEATSTONE bridge circuit. Any difference in the composition of the air between the two compartments causes an inbalance in the bridge, which is recorded directly.

The amount of water, evaporated from 1 cm^2 of sweating skin per hour in studies of sweat secretion by ADAMS et al. (1963), is between 4 and 40 mg. The amount of water, evaporated from 1 cm^2 non-sweating skin per hour in studies of the insensible perspiration of forearm skin, is about 0.4 mg. In studies of sweat secretion, a quick response to any change in the rate of evaporation is an important factor; in studies of insensible perspiration the sensitivity of the equipment is more important. In both studies a thermal conductivity cell can be used as a sensor, though the dimensions and characteristics of the cell have to be chosen in accordance with the object in view. So, ADAMS et al. (1963) used a GOW MAC macro cell in their studies of sweat secretion, and SPRUIT (1967, and this chapter) have applied a GOW MAC micro cell for the measurement of insensible perspiration.

The air flow recommended for the thermal conductivity macro cell used by ADAMS et al. (1963), is 100 ml/min, and the air flow actually used by these investigators was 200 ml/min. The air flow recommended for the thermal conductivity micro cell used by SPRUIT (1967) in the measurement of insensible perspiration, is 5 - 15 ml/min, and the air flow in fact used was 10 ml/min. As a consequence, the humidification of the air in the application of the micro cell was 20 times as great as in the macro cell. With equally sensitive thermistors used as sensitive elements in both methods, the resulting sensitivity of the total thermal conductivity micro cell is thus 20 times that of the macro cell.

ADAMS et al. (1963) have commented, that the latency and time constant of response of the unit depends on the size of the sampling cup, the length and diameter of the tubing connecting to the cell, and the air flow rate through the system. Using a 4.7 ml cup for sampling and approximately 60 cm tubing with an air flow of 200 ml/min, ADAMS et al. found a response latency and time constant in the order of 2 sec and 10 sec for cup and tubing respectively. The reduction of the dimensions of the cup in the measurement of the insensible perspiration (0.4 ml volume of the 1 cm^2 cup) compensates for the increased lag time resulting from the smaller flow of 10 ml/min, so that it is only of the order of 3 sec as far as the cup is concerned. Use of teflon tubing with an inner diameter of only 1.5 mm is possible because of the smaller air flow, so that the time lag of 100 cm tubing is still limited to 10 sec.

In the measurement of sweat secretion it is not very important whether dried air or another gas, e.g. environmental humid air is used. Dried air is preferable for sweat measurement because it certainly allows an appreciable amount of humidification, and will not become saturated. In the measurement of insensible perspiration, on the other hand, it is desirable to use

the ordinary environmental humid air. The permeability - and hence the water vapor loss - of the horny layer of skin are changed by varying the water content of the horny layer. Such changes are of physiological and medical interest, but have the disadvantage that they complicate the measurement. On the other hand, measurement in humid air is carried out rapidly, because the skin will not need time to be acclimatized to a different humidity if the normal environmental air is used. This implies that measurements in environmental humid air can be read instantaneously, whereas measurements in dry air may require some time for equilibration.

It can be calculated that the air after passing over the skin will never become saturated in normal environmental circumstances (see Figure IV.3), so that the introduction of dried air in the measurement of insensible perspiration is not necessary from this point of view.

II.2 METHOD OF MEASUREMENT IN ENVIRONMENTAL AIR

The equipment for measuring the water vapor loss from human skin in environmental air consists of:

1: a thermal conductivity cell or katharometer block, GOW MAC micro cell JDC 133, this being a sensitive water detector,
2: an air pump, "Reciprotor" electromagnetic piston pump, delivering a constant air flow to the cell,
3: two needle valves,
4: a 3- or a 4- channel cock,
5: two flow-meters, e.g. "Rotameters", indicating 3 - 30 ml/min
6: teflon tubing, external diameter 4 mm and internal diameter 1 - 1.5 mm,
7: stainless steel tubing, external diameter 2 mm,
8: a 1 cm^2 x 4 mm capsule for sampling the water vapor from the

skin,

9: a 30 ^{o}C thermostat,

10: an 8 V accumulator, being a direct current power supply,

11: 0 - 10 mA meter, controlling the total electric current through the thermistors at 6.00 mA,

12: 0 - 2.5 mV pen recorder,

13: 5120 ohm decade stepping switch resistor, used as a sensitivity control,

14: two 500 ohm resistors, for balancing the WHEATSTONE bridge,

15: 500 ohm variable resistor, for setting the bridge current,

16: 100 ohm 10-turn "Helipot" resistor, for zero adjustment of the bridge,

17: two 50 ohm resistors, for counterbalancing the "Helipot" 100 ohm resistor in the bridge circuit.

18: a thermocouple element, for measuring the skin temperature inside the capsule,

19: an aspiration hygrometer, for measuring the humidity of the environmental air.

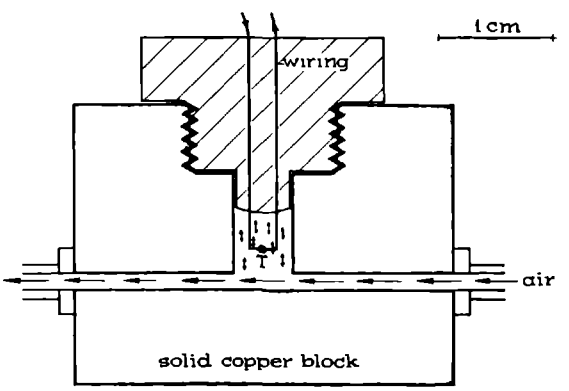


Figure II.1

Cross section through a compartment of the GOW MAC thermal conductivity micro cell JDC 133.

A cross section through the thermal conductivity cell used in our measurements is represented in Figure II.1. The thermistor (T) is embedded in glass. The sample and reference air streams flow just below the thermistor beads. Two thermistors are mounted in one block (see Figure II.5). The thermistor is heated by an electric current of 3 mA; its temperature is about 80 ^{o}C, whilst the atmosphere around the solid copper block is kept at a constant temperature of 30 ^{o}C, obtained by circulating thermostatted water through a spiral tube mounted in the heat

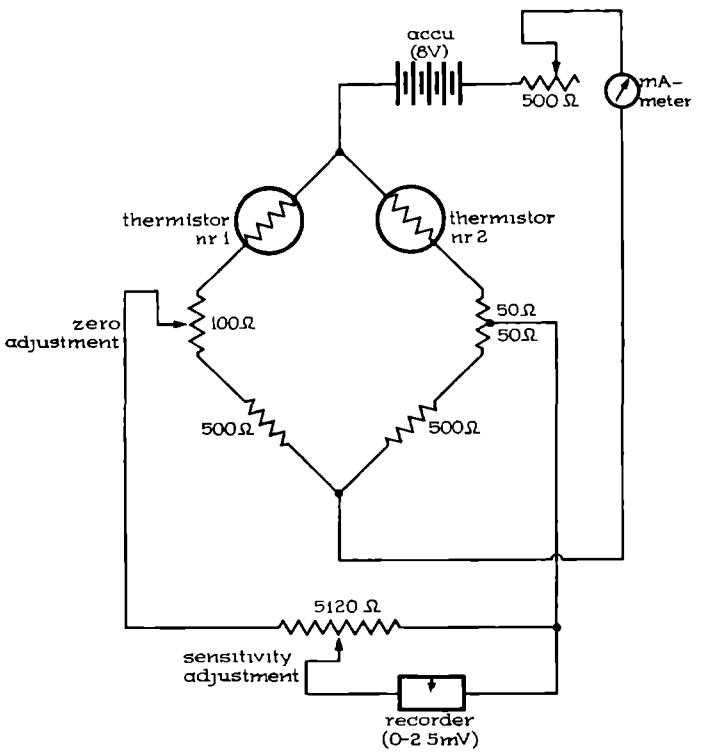


Figure II.2

The WHEATSTONE bridge circuit for the measurement of the change of the electrical resistance of the thermistors of the thermal conductivity micro cell.

insulated container. Most of the heat, produced in the thermistor by the electric current, is conducted through the air to the solid copper block. The rate of this conduction is determined by the thermal conductivity of the air, and this thermal conductivity is dependent on the composition of the air which is passing through the channel.. The rate of flow is 10 ml/min.

The electrical resistance of the thermistors is very much dependent on their temperature and, because of this, is used as a measure of the thermal conductivity and the composition of the air. The thermistors of a katharometer block are a matched pair, and the difference between their electrical resistances is determined by means of a WHEATSTONE bridge circuit (Figure II.2). The electric power is supplied by an 8 V accumulator and the current is set at 6.00 mA by means of a variable 500 ohm resistor. The circuit is basically that suggested by the thermal conductivity cell manufacturers (GOW MAC Instruments Co., Madison, N.J., U.S.A.), and the 6.00 mA current is recommended by them. The 8 V accumulator direct current power supply is the only real modification in the recommended circuitry. The output from the bridge is set at "zero" by means of the variable 10 turn "Helipot" resistor of 100 ohm. As soon as the resistance of one of the thermistors is changed, an electric current will be recorded. An output of one scale unit (0.025 mV) is cancelled out by a zero adjustment of 0.007 ohm. The effective sensitivity of the 2.5 mV recorder can be decreased by a decade stepping, discontinuously adjustable switch resistor of 5120 ohm.

If measurements of the water vapor loss of the skin are wanted both in dry air and in environmental humid air (as has been the case in our investigations), both dry and humid air have to be immediately available. The scheme for the preparation of a stream of dry or environmental humid air at choice is presented in Figure II.3. An electromagnetic piston air pump deli-

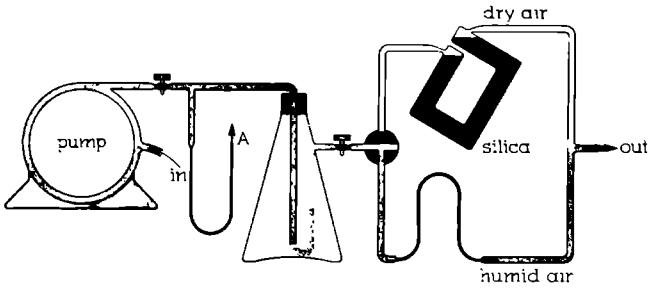


Figure II.3

Diagram for the preparation of a 30 ml/min stream of dry air or environmental humid air.

vers a stream of about 300 ml environmental air per minute. Only one tenth part is conducted into the Erlenmeyer flask, nine tenths being allowed to escape through a capillary tube at A. The resulting stream of 30 ml air per minute, leaving the Erlenmeyer flask, is at constant flow and is conducted either through a tube filled with silica gel or through a capillary tube. The flow resistance of the capillary tube is chosen to be identical with the resistance of the silica gel tube, so that the change over from dry air to humid air (and the reverse change) passes off smoothly. When the drying power of the silica gel becomes insufficient, the tube filled with silica gel has to be dried at the relatively high temperature of 240 ^{o}C (instead of the normally applied 120 ^{o}C), passing dry air or dry nitrogen from a cylinder slowly through the tube during the last hour of the drying process. This yields a drying capacity of the silica gel tube to about 10 ppm water per volume air (less than 0.1 % RH). A well regenerated tube will last for several months' continual service without any appreciable increase of the relative humidity of the air delivered. Closing of the capillary tube at A (see Figure II.3) increases the flow considerably. By closing this tube

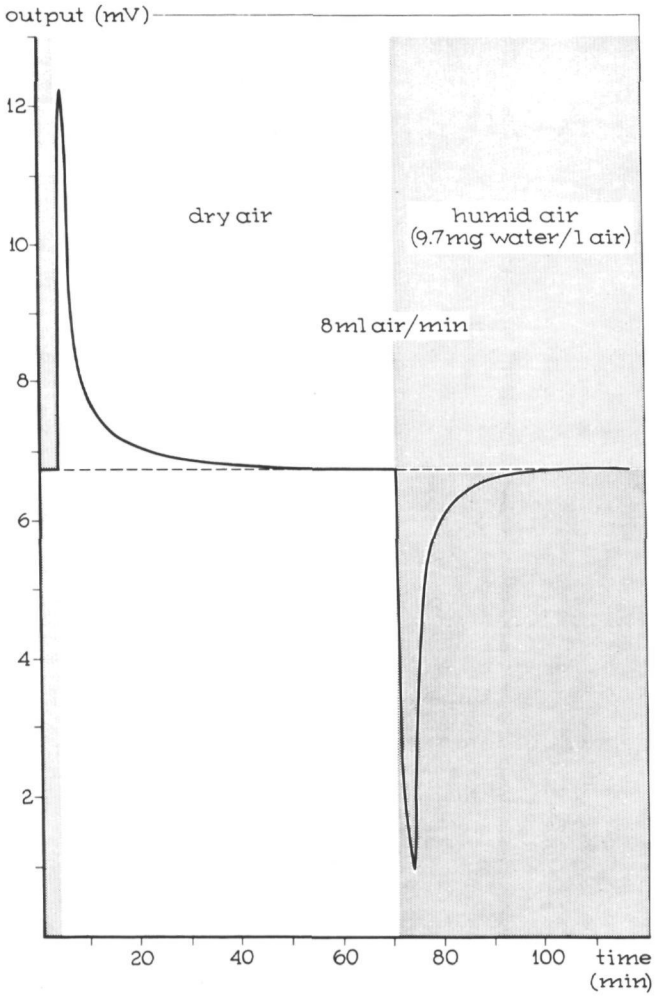


Figure II.4

Recording of the output of the thermal conductivity cell when in a closed system the stream of environmental humid air is switched over to dry air (and the reverse).

with the thumb for about a minute, the complete instrument is quickly ventilated. A normal change from dry air to humid air (and the reverse) will require about 30 - 50 minutes (see Figure II.4). By the "thumb closing" procedure this time can be shortened to about 20 minutes. From Figure II.4 it is also evident that the zero output of the instrument is independent from the humidity of the air used.

The flow diagram of the air through the thermal conductivity cell and over the skin is illustrated in Figure II.5. The air, entering the system at "in", is divided into two parts. One part passes through the first compartment of the thermal conductivity cell, both in diagram "zero" and in diagram "measure". From this first cell compartment the air flow is directed through the stainless steel tube S1 into the three-channel cock which is shown in the centre of both flow diagrams; the connections of the tubes conducting the air flows are interchanged by this cock. When the cock is set to "zero", the air flow from tube S1 is led, via tube S4, into the second compartment of the thermal conductivity cell. In this way the composition of the air is left unchanged; the pathway along the skin surface is short-circuited. The electrical resistances of the thermistors nos. 1 and 2 are balanced in the WHEATSTONE bridge circuit. The air flow is measured by the lefthand flowmeter when it leaves the second cell compartment.

When the cock is set to "measure", the air flow from tube S1 is led into tube S2. The composition of the air remains unchanged until it reaches the water vapor sampling cup which has been placed on the skin. The water evaporating from the skin humidifies the air passing through this cup; the increased water content of the air is represented in the flow diagrams by the blackened areas of the tubes, cup, etc.. The air with increased water content passes via tube S3, the three-channel cock and tube S4 to the second compartment of the thermal conductivity

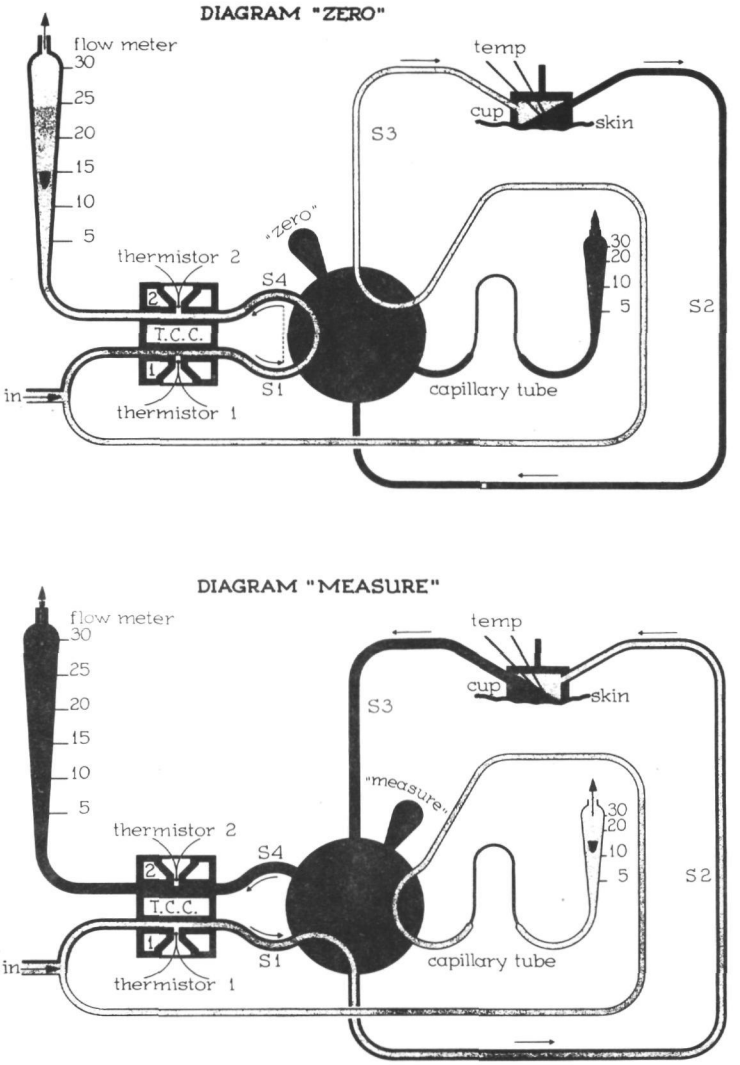


Figure II.5

Flow diagrams of the air stream through the thermal conductivity cell (TCC) and over the skin.

(modified Figure, originally published in J. appl. Physiol. 23: 994-997, 1967)

cell, and leaves the apparatus by the lefthand flowmeter. The composition of the air has been changed, in contrast to the situation of flow diagram "zero", because in this case the air passing through the second cell compartment has been increased in its water content. Consequently the electrical resistance of thermistor no. 2 is altered.

However this is not the only change influencing the resistance of thermistor no. 2. The rate of the air flow has been decreased by changing the position of the three-channel cock from "measure" to "zero"; this also influences the conduction of the heat from the thermistor to the katharometer block, and hence the electrical resistance of the thermistor. A decrease in the rate of the air flow cannot be avoided when a three-channel cock is used, because the flow resistance through the long and narrow tubes S2 and S3 and through the cup is always higher than through the shortcircuited path shown in diagram "zero". This change could be avoided if a four-channel cock were used and an additional tube connected with the same flow resistance as tubes S2 and S3 together. This solution of the problem is, however, unnecessary, and another solution has been preferred.

According to flow diagram "zero", tubes S1, S4, and also S3 contain non-humidified air. Immediately after switching the three-channel cock from "zero" to "measure", the non-humidified air from tubes S4 and S3 starts passing through the second compartment of the thermal conductivity cell; this continues for about 20 seconds. The rate of its flow is exactly the same as the rate of flow of the humidified air which will follow, and the output of the WHEATSTONE bridge circuit during this 20 seconds passage of non-humidified air can be recorded and compared with the output of the bridge circuit during the subsequent period of measurement. The change of this output is thus only dependent on the change of the composition of the air, because measurements are made whilst the flow rate remains constant. An exam-

ple of a measurement, demonstrating these effects, is shown in Figure II.11, the recording of a calibration experiment.

It has been mentioned before that the measurement of the water vapor loss of human skin in environmental humid air is useful because in this way the skin need not be acclimatized to an altered humidity. Therefore, the air above the skin (contents of the cup) must not be allowed to become saturated with water. In order to realize this, a part of the air flow entering the system at "in", is directed through tube S3 (diagram "zero"), through the cup, tube S2, a capillary tube, and the righthand flowmeter. The length of this capillary tube has been chosen so as to create a flow of 10 ml/min as indicated by the righthand flowmeter when the cock is set to "zero"; this is the same flow as is indicated by the lefthand flowmeter when the cock is set to "measure". A fine adjustment of the flow rate is provided by a needle valve between the capillary tube and the righthand flowmeter. As a result, both in settings "zero" and "measure", the air flow through the cup is identical: 10 ml/min. The air flow is only stopped for a very short moment when the three-channel cock is changed from position "zero" to position "measure", less than one second.

The length of the stainless steel tube S4 may be kept short, but the length of teflon tubes S3 and S2 has to be at least 40 cm for ease in manipulating the cup for sampling the water vapor loss from the skin. The length of tube S3 has therefore been adjusted so that a change of the three-channel cock from position "zero" to position "measure" was followed by a 20 seconds passage of non-humidified air through the second compartment of the thermal conductivity cell. The length of tube S2 is also an important characteristic of the speed of the measurement; it is best chosen to be about 10 to 20 cm longer than tube S3. The reason of this choice and its effect can be described as follows.

The air flow through tubes S2 and S3, when the cock is set

to "measure", is in the opposite direction as compared to the flow through these tubes, when the cock is set to "zero". According to the flow scheme of diagram "zero", the tube S3 contains air of unchanged composition flowing through the sampling cup. The humidified air leaves the sampling cup through tube S2. Immediately after switching the three-channel cock to the position "measure", the humidified air contents of tube S2 change flow direction and return to the sampling cup, are again moistened by water vapor from the skin, and after being twice humidified, reach the second cell compartment of the katharometer via tubes S3 and S4.

In the meantime another phenomenon causes a decrease of the water content of this double-humidified air so that it reaches the second cell compartment at only a very slightly increased level above the once-humidified air. The flow in the middle of the narrow tubes is fast, but the flow near the walls of these tubes is much slower. Near the walls of tubes S3 and S4 some non-humidified air adheres, therefore, whilst the double-humidified air starts to pass through the middle of the tube. As the excess water content of the double-humidified air is exchanged to some extent with the lower water content of the non-humidified air at the walls, the double-humidified air will not reach the second cell compartment as such. Its water content will be decreased considerably; this is dependent on several factors, including the length and diameter of tubes S3 and S2. Curve a of Figure IV.4 represents an example of these effects, demonstrating a small "overshoot" resulting from this double-humidification of the contents of tube S2. Curve a shows that the time of the measurement can be reduced to less than two minutes by an appropriate choice of the dimensions of the tubes.

Stainless steel was selected for the material of the short tubes S1 and S4, the sampling cup and other parts in order to minimize absorption of water vapor at the walls and diffusion of water

vapor through the walls (WALKER & CAMPION, 1965). The material has to be internally clean as otherwise the time of indication of the result of a measurement is increased by absorption and exchange of water vapor by the pollution at the walls. The katharometer block was made from copper. Although this material is slightly inferior as far as its use in a water vapor measurement equipment is concerned, the results were satisfactory and therefore this block was not exchanged for a stainless steel block. As one has to be able to manipulate the sampling cup very easily, and the pressure of the cup on the skin must not be allowed to become too great - especially at injured skin sites - stainless steel or nickel tubing cannot be applied in the tubes S2 and S3 which connect the sampling cup to the instrument. Teflon tubing was chosen because this material has, as far as is known, the smallest permeability to water vapor and the lowest absorption of the various plastic materials available. The walls of the teflon tubes S2 and S3 have to be rather thick (about 1.0 mm) in order to prevent diffusion of water from the outside atmosphere, especially when dried air is used. Walls of 0.2 mm thick teflon proved to be unsatisfactory.

The output of the WHEATSTONE bridge circuit is registered by a 0 - 2.5 mV recorder. The change of the composition of the air determines the output of the recorder (mV). The increase of the water content of the air, originating from its passing over a fixed area of human skin or another source, is inversely proportional to the flow of the air (ml/min). Therefore, the amount of water vapor lost from the skin is calculated by multiplying the output of the recorder (mV) by the flow, determined by the left-hand flowmeter (ml/min). 1 mV ml min^{-1} appeared to be equivalent to about 90 µg water per hour.

The accuracy of the reading of the flowmeter ("ROTAmeter" 3 - 30 ml/min, ROTA, Oeflingen, Baden) is 0.1 ml/min. The flow will usually be constant for some hours, but in some circum-

stances it will tend to decrease or increase slightly, especially for a short time after the setting has been changed or after the instrument has been put into use. The reading of the "zero" output of the thermal conductivity cell varies slightly with the flow rate. The variation depends on the balance between the matched pair of thermistors and the precision of assembling the thermistors in the block. The dependency, determined for a commercially obtained thermal conductivity GOW MAC micro cell JDC 133 block, is represented in Figure II.6. The value of the maximum slope is 0.005 mV per flowrate change of 0.1 ml/min. This deviation cannot be read on the 2.5 mV recorder and is too small to be of significance in the measurement of the water vapor loss.

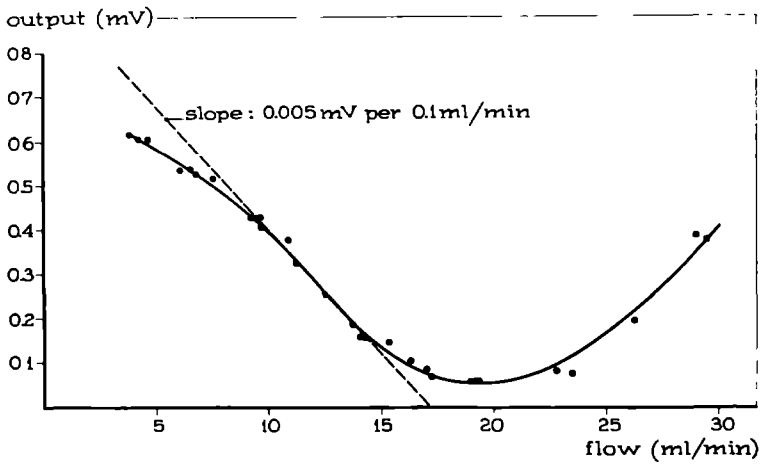


Figure II.6
Variation of the output of the thermal conductivity cell with the flow rate of the air.

A cup of 1.0 cm^2 is the largest one that can be applied in measurements on the fingers (Figure II.7). It is also the largest one that can be used without precautions being taken for effective convection over the investigated skin area. The thermocouple

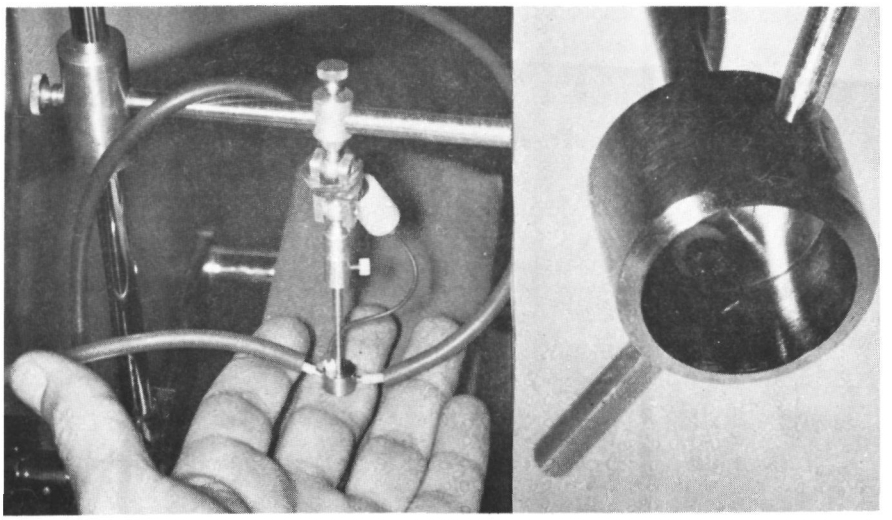


Figure II.7

A 1.0 cm^2 cup, incorporating a thermocouple element, used on the finger.

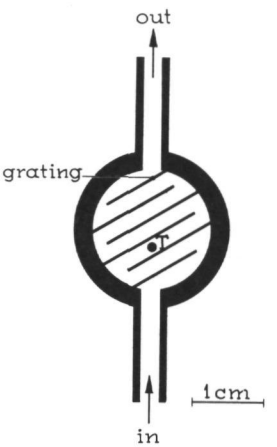


Figure II.8

The convection of the air stream over the skin under a 2 cm^2 cup is improved by partitions, mounted inside the cup. A thermocouple element is installed at T.

element for measuring the temperature of the skin inside the cup is mounted in the middle of the cup, so that it touches the skin and will leave behind a slight indentation shortly after the measurement. In a larger, 2 cm^2 cup, partitions have been mounted in order to improve the contact of the air stream with the skin surface (Figure II.8). Cups of only 0.2 cm^2 are preferably used in measurements on sweating skin.

II.3.1. CALIBRATION

The non-linear relation between the water concentration of the air and its thermal conductivity was already published by GRUSZ & SCHMICK in 1928. They not only investigated water/air mixtures, but many other gas mixtures too. However, the deviating behavior of the water/air mixture was most striking and so it became the example of non-linearity, and impeded further developments; wrongly as has been argued by CHERRY (1965).

The thermal conductivity of pure water vapor is relatively low. This value is called the "polar" thermal conductivity (see Table II.A), as the collisions between molecules occur only between polar water molecules. With a mixture containing only a small proportion of polar water vapor and a large proportion of nonpolar air molecules, the mutual interaction between polar water molecules is greatly reduced since the polar molecules collide mainly with nonpolar air molecules. The contribution of the water vapor to the thermal conductivity of the mixture does not relate to the pure "polar" thermal conductivity of water vapor. At very low water concentrations this contribution is determined only by the value of the "nonpolar" thermal conductivity of water vapor; this value is considerably higher than the value of the polar thermal conductivity of water vapor and the value of the thermal conductivity of air; see Table II.A.

The thermal conductivity of air containing 45 vol.% water

Table II.A:
Values of thermal conductivities (TC) of water vapor and of air.

	Thermal conductivity (TC) at a temperature of: 65*)	200**) °C
TC of air:		3.7 $\mu W\ m^{-1}\ ^{\circ}C^{-1}$
"polar" TC of water vapor:	2.07	2.85 $\mu W\ m^{-1}\ ^{\circ}C^{-1}$
"nonpolar" TC of water vapor (low concentration):	3.14	7.5 $\mu W\ m^{-1}\ ^{\circ}C^{-1}$
ratio "nonpolar"/"polar" TC:	1.5	2.6

*) according to BENNETT & VINES (1955)
**) according to CHERRY (1965)

vapor is identical with the thermal conductivity of pure dry air; and the maximum thermal conductivity of air/water-vapor mixtures exists in air containing about 20 vol.% water vapor (GRUSZ & SCHMICK, 1928). Of course such high water vapor concentrations can only be realized under non-physiological circumstances; in normal environmental conditions they are not possible. Even at temperatures in the order of 20 - 30 °C, air is completely saturated with water vapor by only 2 - 3 vol.% water (Figure II.9, curve at 100 % RH). Therefore, only the relation between the thermal conductivity of air and water vapor concentrations up to 3 vol.% is strictly relevant to our investigations.

The relative thermal conductivity of mixtures containing low concentrations of water vapor in several different gases was investigated by CHERRY (1965). Some of these results are presented in Figure II.9. The thermal conductivity of water vapor mixtures with argon, with carbon dioxide and with oxygen was found

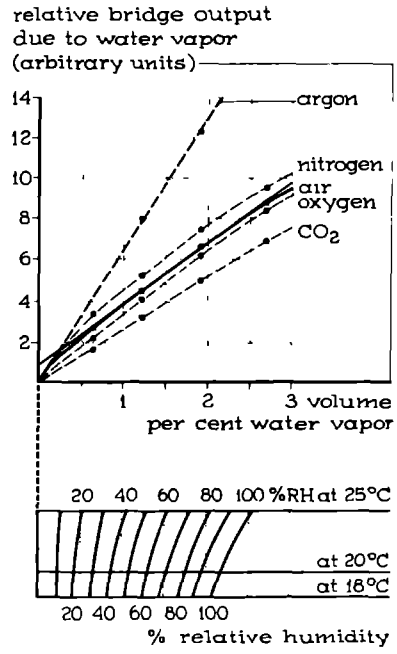


Figure II.9

The relative thermal conductivity of mixtures of water vapor with several gases (Ar, N_2, O_2, air, and CO_2) as published by CHERRY (1965). The water concentrations of CHERRY's Figure are additionally compared to the relative humidity values at normal temperatures (18°, 20° and 25° C) as represented at the foot of the Figure.

to follow a linear relation to the water concentration. Only the thermal conductivity of water vapor mixtures with nitrogen - and, as a consequence also with air - was found to deviate from this linear relation to the water concentration. The non-linearity is especially relevant at concentrations of 0 0 - 0.6 vol.% water; at concentrations between 0.6 and 2.5 vol.% a linear relation can be assumed as is evident from the straight unbroken line drawn through CHERRY's measurements of water/

/ air mixtures. The slope of this graph between 0.0 and 0.2 vol.% water appears to be about twice that found at concentrations between 0.6 and 2.5 vol.% water. This means that the effective sensitivity of the thermal conductivity cell will be twice as much in dry air (0.0 - 0.2 vol.% water) as in environmental humid air (about 1.5 vol.% water).

From the foregoing it is apparent that the thermal conductivity cell be calibrated both in dry and in environmental humid air.

Several methods (too many) have been proposed for the calibration of thermal conductivity cells and other hygrometrical instruments of this kind. In the same year (1948) WEXLER improved the method of WALKER & ERNST (1930) and CHERRY improved his own former method, which he "discarded, because of er-
"ratic results obtained."
"Reproducible calibration is a difficulty common to all relative
"humidity or water vapor pressure-sensing elements, arising in
"part from the detection characteristics of the individual unit,
"but largely related to difficulties in obtaining stable, predicta-
"ble, low levels of water vapor." (ADAMS et al., 1963).

II.3.2 DIRECT CALIBRATION METHOD

The problem of obtaining stable and controlled rates of evaporative water loss was met by ADAMS et al. (1963) by running 200 ml dry air per minute through a brass block containing water in the liquid phase. Fluctuations of the rate of evaporative water loss were less than 0.03 mg water per minute. However, the rate of water vapor loss by insensible perspiration from one cm^2 forearm skin as measured with our thermal conductivity cell method is only 0.5 mg water cm^{-2} h^{-1}. The rate of water production in the calibration device is, therefore, limited to only 0.5

mg water per hour, that is 12 mg water per day. In order to reach this requirement, a simple calibration bottle has been devised (Figure II.10). The air flow through the bottle passes over an opening, A, a small area (1 mm^2) of air moistened by wetted silica gel in the interior of the bottle. The water loss from this bottle is about 0.5 mg/h. As the accuracy of an ordinary analytical balance is about 0.2 mg, a calibration experiment should preferably be extended over about two days, resulting in about 25 mg total water loss. The calibration bottle is weighed before the calibration experiment starts and at the end of the experiment.

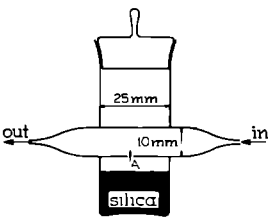


Figure II.10
Glass bottle for calibration of the thermal conductivity cell water analyzer.
(reprinted from the J. appl. Physiol. 23: 994-997, 1967)

The water loss from the bottle is governed by FICK's law of diffusion. In accordance with FICK's law the rate of the water loss through the opening A was shown only to be dependent on:

1: the area of the opening A;
2: the temperature;
3: the difference between the humidities of the air on either side of opening A, i.e. between the interior of the bottle above the moistened silica gel and the air running through the 10 mm diameter tube.

It has been shown to be independent of the flow rate of the air through the tube between 3 and 30 ml/min.

The water loss from the calibration bottle, linked into the measuring circuit in place of the cup (Figure II.5), is not influenced by a shift of the three-channel cock from "zero" to "measure". Thus, a determination of the water content of the air can be performed as frequently as is wanted by the investigator during a calibration experiment, without disturbing it. The investigator frequently wants to check whether the rate of moistening of the (preferably environmental humid) air by means of the calibration bottle is constant, as it is difficult to prepare, with a high degree of certainty, a source of humidification which will remain stable for a calibration period of two days. In this respect, the factors mentioned as 2 and 3 in the foregoing paragraph (the temperature and the humidity gradient) are particularly important. The calibration bottle can be placed in a thermostat if the temperature of the room fluctuates more than $\pm$ 1 ^{o}C. It has been calculated that variations of the temperature of the calibration bottle of 1 ^{o}C will cause variations of the water content inside the bottle of about 6 %.

The water content of the environmental humid air entering the calibration bottle can be supplied from a 200 l tank if extreme reproducibility is wanted. In practice it proved sufficient to obtain the air directly from the outside (the room) through a 1 liter bottle as shown in Figure II.3, periods of stable weather being chosen for the calibration experiments. Every two hours (or at least every four hours) during a calibration experiment a series of at least 5 measurements of the water content of the air is made, and the average of these 5 values of the humidification of the air calculated. In this way about 24 (resp. 12) results are obtained during a calibration experiment over a 2 days period. A reliable result of the calibration experiment is found by calculating the mean of the 24 individual values. In a typical experiment no result deviates more than about 10 % from this mean.

In normal calibration experiments the silica gel inside the calibration bottle was moistened with about 50 % water in order to maintain a considerable humidity in the interior of the bottle, so that a sufficient difference of the water content of the air at both sides of the opening A is reached during the experiment. Unfortunately it is difficult to establish the precise relative humidity inside the bottle when silica gel is used. In certain experiment this is a disadvantage, and therefore in some experiments 20 % glycerin solution was substituted for the silica gel. The relative humidity of the air above 20 % glycerin is known to be 95 % and the humidity of the air inside the bottle will have reached this value. Similarly saturated NaCl solution was used in other experiments, yielding 75 % RH in the interior of the bottle; or 51 % sulfuric acid, yielding 35 % RH. FICK's law proved to be valid.

When the normal environmental humid air from the room is chosen as a source of the air flow, the variation coefficient (relative standard deviation) of a series of 10 measurements - in total requiring 20 minutes - is 2 %; the variation coefficient of a series of 35 measurements - in total requiring about 50 min - is 3 %, somewhat larger because of slight variations of the humidity of the environmental air. The variation coefficient of the means of 12 series (each consisting of 10 measurements) during a calibration experiment of 2 days has also been 3%. When temperature fluctuations during such experiments were about 1 ^{o}C, the variation coefficient was increased to about 5 %. This latter variation coefficient has been used as a check of the reliability of the calibration experiment.

When dried air, containing 0.06 vol.% water, is taken as a source of the air flow, the variation coefficient of a series of 5 measurements - in total requiring considerably more time than measurements in humid air, namely about 50 minutes - is 3 %.

These variation coefficients of the calibration experi-

ment measurements of 2 %, 3 %, or 5 %, are considered to be acceptable, as the variation coefficient of measurements of the water vapor loss of skin in vivo by an electrolytic water analyzer (SPRUIT & MALTEN, 1965) was found to be about 10 %, when measurements were taken at several different days during a monthly period. This appreciable variation coefficient must be attributed to physiological changes of the water vapor loss of human skin, as the variation coefficient of measurements on cadaverous skin is only 2 %.

The flow through a GOW MAC thermal conductivity micro cell JDC 133 must remain within the limits of 5 and 15 ml/min for technical reasons, as is stated by the manufacturer. A typical uptake of water by an air flow of 10 ml/min from 1 cm^2 skin (water vapor loss 0.5 mg cm^{-2} h^{-1}) or from the calibration bottle (figure II.10) is 0.83 mg water per liter air; an increase of 0.11 vol.% water. This increase is sufficiently small for it to be possible to check the conclusion based on the results reported by CHERRY (1965, see Figure II.9), that the sensitivity should be twice as high in dried air (0.0 - 0.2 vol.% water) as it is in humid air (1.5 vol.% water). Some calibration experiments have been carried out for this purpose. In these experiments dried air with 0.06 vol.% water (determined by an electrolytic water analyzer) and environmental humid air of 1.5 vol.% water (determined with the aid of an aspiration psychrometer) was used. The results are given in Table II.B (see in Section II.3.4), and do not confirm the above conclusion.

II.3.3 INDIRECT CALIBRATION METHODS

The direct calibration method is laborious as it requires many measurements over a period of two days. Other methods were, therefore, developed to check the sensitivity of the

instrument at any given moment. One of the methods is suitable to calibrate the output of the instrument when dry air is used as a carrier gas; the other one can be used when the calibration of the output of the instrument is wanted in environmental humid air as a carrier gas.

In the calibration against an aspiration psychrometer a plastic 10 liter bottle is filled with environmental air. The water content is calculated from the measurement of the humidity by the psychrometer. A flow of dried air is directed through the thermal conductivity cell according to diagram "zero" (Figure II.5) for one hour. The cup is replaced by the plastic bottle and the calibration measurement is started by switching the three-channel cock to "measure". The output of the thermal conductivity cell is recorded (Figure II.11).

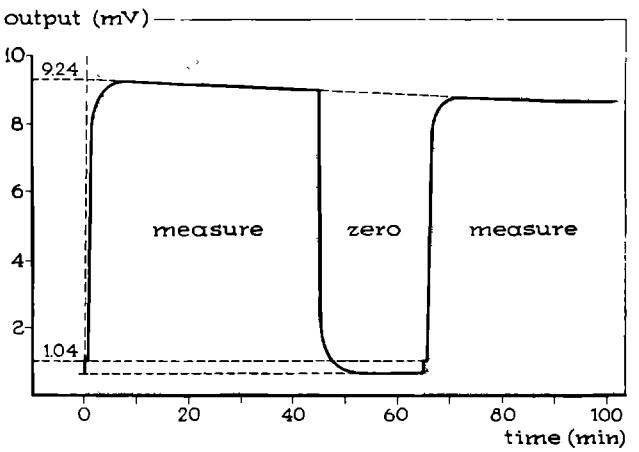


Figure II.11

Recording of the thermal conductivity cell water analyzer during a calibration against an aspiration psychrometer, with the use of environmental humid air, containing 12.6 mg water per liter air, from a plastic 10 liter bottle, as described in the text. The contents of the bottle is replaced by a flow of 8.0 ml dried air/min. (reprinted from the J. appl. Physiol. 23: 994-997, 1967)

All measurements using dried air (0.5 mg water per l air) as a reference gas take some 10 minutes before a steady state has been reached. As the water concentration of the contents of the plastic bottle decreases during the calibration by the mixing with dried air (8.0 ml/min), the output of the thermal conductivity cell decreases and the extrapolated output at the beginning of the calibration must be calculated, being in Figure II. 11: 9.24 - 1.04 = 8.20 mV. The increase in the water concentration of the air passing the thermal conductivity cell is known from the readings of the aspiration psychrometer and the measurements of the water content of dried air (analyzed with an electrolytic water analyzer), being in Figure II.11: 12.6 - 0.5 = 12.1 mg water per liter air. The sensitivity of the thermal conductivity cell is calculated from the results of these measurements, being, in the example shown in Figure II.11: 8.2 / 12.1 = 0.68 mV (l air) $(mg\ water)^{-1}$.

This calibration experiment has been carried out with a flow of dried air (0.06 vol.% water) through the first compartment of the thermal conductivity cell. The second compartment contains environmental humid air (1.5 vol.% water). According to CHERRY's graph (Figure II.9) a measurement carried out in this way should deviate only 5 % from that obtained when humid air (1.5 vol.% water) also flows through the first compartment. Thus the measurement effectively represents a calibration in humid air.

In an electrolytic water analyzer the water is absorbed by P_2O_5 and electrolyzed by an electric current. The method has been applied in the measurement of the water vapor loss from human skin (SPRUIT & MALTEN, 1965). The method can only be applied if well dried air is used.

As the water is decomposed by the electrolytic water analyzer and not decomposed by the thermal conductivity cell, the moistened air is led through the thermal conductivity cell be-

fore it passes into the electrolytic water analyzer. The air flow is finally measured at the exit.

The output of the thermal conductivity micro cell JDC 133, which was used, is not completely pressure-independent. The output increased by 1 % when the pressure of the air was increased by 1 mm Hg. The deviation is dependent on both construction factors and also on the way of mounting. The increase of the thermal conductivity, according to the formula of VINES & BENNETT (1954), should be 0.002 % instead of 1 % under these circumstances. This pressure dependency does not disturb normal measurements as the difference of the pressure between the first and the second thermal conductivity cell compartment is somewhat less than 0.1 mm Hg. When an industrial electrolytic water analyzer is mounted in series with the thermal conductivity cell in a calibration measurement, however, care should be taken that all valves increasing the air pressure have been removed from the water analyzer, as otherwise the output of the thermal conductivity cell and its effective sensitivity will be changed.

The water concentration of the air flow must be increased only very slightly. This increase is conveniently obtained from a calibration bottle (Figure II.10) or from a flow through a cup of 2 cm^2, placed at cadaverous human skin in vitro. Recombination of H_2 and O_2 (see JONES & PETERSEN, 1965) did not occur in the electrolytic water analyzer, as has been checked by varying the flow from 30 to 3 ml/min.

II.3.4 CALIBRATION RESULTS

The sensitivity of the GOW MAC thermal conductivity micro cell JDC 133 which was used in this work was found to be very close to 0.69 V (l air) $(g\ water)^{-1}$ according to all methods (see Table II.B).

Table II.B

Sensitivity of a GOW MAC thermal conductivity microcell JDC 133, used as a water analyzer; results of some calibration methods.

Calibration method; no. and method	Air, supplied to instrument	Rate of moistening (mg water per hour)	Sensitivity in V (l air) (g water)$^{-1}$
$a_{1.1}$ Direct; (weight of water)	dried air	0.5	0.68
$a_{1.2}$ Direct; (weight of water)	dried air	8	0.69
a_2 Direct; (weight of water)	environmental humid air	0.5	0.67*)
b_1 Indirect, against an electrolytic water analyzer	dried air	0.5	0.70
b_2 Indirect, against an aspiration psychrometer	dried air	8	0.68

*) Average measurement of 0.61, corrected by 10 % owing to a slow increase of the output of the instrument (see second paragraph of Section II.3.4)

The results of the direct calibration method a_2 with application of environmental humid air originally seemed to deviate 12 % from the other results. Though small, this difference appeared conspicuous as three determinations of the sensitivity by this method a_2 yielded respectively 0.60, 0.61 and 0.62 V (l air) (g water)$^{-1}$. The error appeared to have been caused by the speed with which these measurements had been performed; the recording was carried out in about one minute, but the output increased about 10 % during the next 10 minutes.

As far as the results as a whole are concerned, it is clear that the sensitivity of this thermal conductivity cell is identical whether dried air was used, whether the air was moistened with a small amount of water vapor (0.5 mg water/h), or with a large amount of water vapor (8 mg water/h). The same results were also obtained regardless of whether a direct method or an indirect calibration method was used. The results obtained with a moistening rate of 0.5 mg water/h (increase of 0.1 vol.% water) (methods nos. $a_{1.1}$ and b_1), might be expected to be double the results obtained by means of the high moistening rate of 8 mg water/h (increase of about 1.5 vol.% water) (methods $a_{1.2}$ and b_2), if CHERRY's graph (Figure II.9) were valid. The results certainly do not confirm the measurements of CHERRY (1965) as far as the non-linearity of this thermal conductivity cell is concerned.

The sensitivity of another GOW MAC thermal conductivity micro cell JDC 133 appeared to be about 20 % lower than the sensitivity of the one used to obtain the results shown in Table II.B. The sensitivity of this cell also appeared to be similar in dry air or in humid air.

II.3.5 SPECIFICITY CHECK

The thermal conductivity cell only indicates a change in thermal conductivity, and hence in the composition, of the mixture. Certainly, it is not a specific water sensor. Therefore, it has to be verified that its specificity is sufficient in any particular application; in this case the measurement and recording of the water vapor loss of human skin.

Measurements using cadaverous human skin cause no problems as far as the specificity of the measurement of the water vapor loss is concerned. The skin is placed upon a grating, the dermis being immersed in a 0.9 % NaCl solution thermostatted at 30

^{0}C. In these circumstances, between the interior and the outside of the skin in vitro, only a water concentration difference exists. Hence, the method of the measurement need not be a specific one.

When measuring the water vapor loss of human skin in vivo, the cup is merely placed upon the skin of the forearm of a volunteer. In spite of this simple situation, it is nevertheless more complicated than the in vitro situation, as water is not the only substance which diffuses through the skin in vivo. About 0.1 mg CO_2 diffuses from 1 m^2 forearm skin in an hour (BARRATT, 1897: ROTHMAN, 1953). An increase of 0.01 mg CO_2 diffusing into 600 ml air can be calculated to cause a decrease of some 0.5 % of the output of the thermal conductivity cell, when using typical forearm skin which is losing 0.5 mg water per cm^2 and per hour. However, certain interfering factors reduce the accuracy of this calculation, for example the formation of hydrates (CLAUSSEN, 1951: KUNZ, 1952: CHERRY, 1965). It is therefore necessary to check the output of the thermal conductivity cell against a specific water sensor when measuring the water vapor loss of human skin in vivo. An electrolytic water analyzer can be used as such, in the same way as in the indirect calibration experiments. The results revealed that the measurements of the apparent water vapor loss by the thermal conductivity cell were increased by 4 to 6 % because of the effects of interfering vapors. This small deviation has been determined by comparing successive measurements in vivo and measurements in vitro. Though small, this deviation represents a systematic error of the measurements of the water vapor loss in vivo. The deviation can be tolerated, however, as the variation coefficient of measurements of the water vapor loss of human skin in vivo by an electrolytic water analyzer has been shown to be about 10 % (SPRUIT & MALTEN, 1965), and the values found may be corrected by about 5 %. The same variation coeffi-

cient has been found in in vivo measurements using the thermal cunductivity cell, both with environmental humid air and with dry air.

CHAPTER III

THE BARRIER TO WATER VAPOR LOSS

Summary

The barrier to water transport is located in the horny layer of the skin, the stratum corneum. It can be removed by "stripping" this horny layer (about 0.02 mm thick) with sellotape. The resistance of the stripped skin is about 0.6 sec/cm and equals the evaporation resistance of albumin solutions.

The resistance of the water barrier of healthy forearm skin to water vapor loss is 150 - 300 sec/cm and equals the resistance of the water barrier of forearm skin to the transport of water in the liquid phase. It is not influenced by the direction in which the water is being transported.

The evaporation resistance of a layer of n-hexadecane has been measured. The n-hexadecane can be used as a reference substance, when the evaporation resistance of other substances is being investigated. The evaporation resistance of white vaseline has been measured (being about 600 sec/cm per 0.01 mm thick layer), because it is widely used in dermatology as an "occlusive" dressing. The "occlusivity" of vaseline is demonstrated by an experiment on normal forearm skin. The vaseline probably does not remain spread uniformly over the skin surface. The influences of this non-uniformity and other interfering factors are discussed.

Discussions concerning certain subjects of this chapter with dr. F.A.J. TALMAN, Brighton School of Pharmacy, College of Technology, have been very valuable to me.

III.1 THE LOCATION OF THE WATER BARRIER OF THE SKIN

The stratum corneum or horny layer is thought to be the most important part of the skin as far as its resistance to water loss is concerned. The connexion between the outside horny cells of the skin is weak. They are continuously shed in small particles in everyday circumstances (GOLDTSCHMIDT & KLIGMAN, 1967). They can also be removed by fixing adhesive sellotape (e.g. TESA-film no. 4101) to the skin and removing the tape some minutes later when the adhesion to the skin has been realized (the so-called "stripping technique"). At the beginning of the stripping procedure about four layers of horny cells, the stratum corneum disjunctum, are removed, being visible on the sellotape as an opaqueness. Following the stratum corneum disjunctum, the underlying stratum corneum conjunctum is removed next by one careful stripping as a compact layer of about four cells thick. This compact layer of horny cells - the inside horny cells, just above the stratum granulosum, the "living" part of the epidermis - is often called the SZAKALL-layer. By refining the stripping technique SZAKALL (1957) prepared in this way a very realistic biological skin membrane in vitro. In order to simplify investigations of the skin's water barrier properties, other investigators have even prepared more homogeneous membranes from the SZAKALL layer, e.g. consisting of a human epidermal phospholipoprotein (CROUNSE, 1965).

For many years the SZAKALL layer was thought to be the real barrier of the skin against water vapor loss, especially because of a Figure published by BLANK (1952). BLANK himself, however, in his Presidential Address at the 26th Annual Meeting of the Society for Investigative Dermatology (1965) critically remarked that his graph did not really prove that the

barrier is located at the SZAKALL layer alone, but most probably comprises the entire stratum corneum. Anyhow, as soon as the stratum corneum has been removed. - and possibly a part of the stratum granulosum, as an ideal separation is very difficult, if not impossible to obtain (JENKINS & TRESISE, 1968) - the whole function of the skin as a water vapor barrier is lost. This conclusion has been based on the experimental observations that:

1: the water vapor loss of forearm skin increases a hundredfold after the removal of the entire horny layer by "stripping",
2: the water vapor loss is identical whether either the horny layer alone or the entire epidermis has been removed, and
3: the water vapor loss of stripped skin (horny layer removed) is approximately equal to the water vapor loss from ordinary water (MONASH & BLANK, 1958; personal observation).

III.2 THE RESULTANT BARRIER OF THE SKIN AFTER STRIPPING THE HORNY LAYER

Formula (3) describes the relation governing the resistance of a layer against transport. A water barrier is a membrane possessing a resistance against water transport; therefore, r quantitates the water barrier properties. When several membrane layers are located in series. i.e. above each other, their respective resistances can simply be added together in order to get the resultant resistance of the total membrane barrier.

This property has been studied extensively by measuring the rate of evaporation of water from water surfaces covered with a monomolecular layer. In these studies the evaporation from a water surface is compared with the evaporation from a water surface covered with a monomolecular layer under exact-

ly the same experimental conditions. Accordingly:

$$r = \left[\frac{(C - C_o)}{M / A\,t}\right]_{film} - \left[\frac{(C - C_o)}{M / A\,t}\right]_{no\text{-}film} \qquad (22)$$

(compare formula 3); A = the area in cm^2; C = the concentration of water vapor in equilibrium with the liquid in g cm^{-3}; C_o = the concentration of water vapor in equilibrium with the desiccant placed above the surface of the liquid in order to measure the evaporation resistance; t = the time in sec; M = the mass of water vapor absorbed by the desiccant in g. In order to determine the resistance r, two measurements are carried out. One is made at a clean water surface (no-film), and the other is made at a water interface covered with a monomolecular layer (film). Similarly the evaporation rates from stripped skin, from normal non-stripped skin, and from normal skin covered with a protective or occlusive layer can be studied. The results of these latter studies can, therefore, be compared with the studies on monolayers.

ARCHER & LA MER (1954) calculated the specific resistance at the surface of pure water against evaporation of the water to be only 0.0019 sec/cm. They considered that in practical and in experimental circumstances, however, the resistance of evaporation to water is considerable, as inevitably the water vapor has to pass an "immobile" layer of still air after evaporation from the liquid/gas interface. The resistance of such a layer is determined by the diffusion coefficient of water vapor through air, D_w, which is given by:

$$\log D_w = 1.75 \log T - 4.921 \qquad (23)$$

according to International Critical Tables, Vol. V, page 62. The resulting rate of water vapor movement has been calculated and is presented in Table III.A for layers of still air of 2 and 3

Table III.A:

Diffusion (D) and resistance against diffusion (r) of water vapor from saturated air into dry air through boundary layers of 2 and 3 mm still air, calculated at 20 °C, 25 °C and 33 °C.

	D_2	D_3		r_2	r_3	
at 20 °C:	75	51	mg cm^{-2} h^{-1}	0.82	1.20	sec/cm
at 25 °C:	105	70	mg cm^{-2} h^{-1}	0.78	1.17	sec/cm
at 33 °C:	170	113	mg cm^{-2} h^{-1}	0.74	1.11	sec/cm

mm thickness. Evidently the resistance of such layers is much more important than the resistance against evaporation from a water surface of 0.0019 sec/cm.

The thickness of the "immobile" layer above a water / air interface may be calculated from the rate of diffusion of water vapor from the surface. Studying the diffusion of water vapor into still air, and using a temperature of 25 °C LANGMUIR & SCHAEFER (1943) measured 72 mg cm^{-2} h^{-1} and ARCHER & LA MER (1954) measured 80 mg cm^{-2} h^{-1}. These figures indicate an "immobile" layer of about 3 mm thickness. Measurements of SEBBA & BRISCOE (1940) of the evaporation rate of water into an air flow of 6 - 40 cm/sec resulted in a value of 125 mg cm^{-2} h^{-1} at 19.5 °C, from which an immobile layer of 1.2 mm can be calculated. Installing our 0.2 cm^2 sampling cup over a water surface layer and using an air flow of about 3 cm/sec (100 ml/min) we measured, applying our electrolytic water analyzer, an evaporation rate of 85 mg cm^{-2} h^{-1} at 24 °C into dry air. In these conditions the immobile layer is therefore 2.3 mm thick. These values can be compared with the results of measurements of the water vapor loss from stripped skin, applying the same instrument, of about 60 - 100 mg cm^{-2} h^{-1} at about 33 °C. From these values the evaporation resistance of stripped skin

is calculated as 2.10 - 1.25 sec/cm. However, the 2.3 mm immobile layer of air at the surface of the stripped skin caused a resistance of 0.85 sec/cm at 33 °C. Therefore, the real specific evaporation resistance of stripped skin to water vapor can be estimated 1.25 - 0.4 sec/cm, averaging about 0.6 sec/cm. For the sake of comparison, the maximal value of the specific resistance for a bovine serum albumin film is 0.54 sec/cm and for pepsin 1.00 sec/cm (BLANK & MUSSELLWHITE, 1968). Stripped skin is always covered with a film of exudate and will contain albumin in the superficial watery liquid. As the estimated value of the evaporation resistance of stripped skin is about the same value as the evaporation resistance of comparable watery liquids, the conclusion is strengthened that stripped skin has no other barrier to water vapor loss.

III.3 THE RESISTANCE OF THE WATER BARRIER OF UN-STRIPPED SKIN

The resistance of normal un-stripped skin can be calculated from the amount of water vapor lost from a unit of surface of the skin per unit of time, when the temperature is known. Normal values of this water vapor loss, for skin at the volar aspect of the forearm, are between 0.4 and 0.8 mg cm^{-2} h^{-1} at a typical skin temperature of 33 °C. These values correspond to a specific resistance against water vapor loss from forearm skin of 300 - 150 sec/cm respectively. Obviously this resistance is located in the entire stratum corneum, as the water vapor loss increases gradually when the skin is stripped. The experimental increase correlates well with the theoretical increase for a homogeneous mass (BLANK, 1965).

The rate of permeation of liquid water through forearm skin, applying tritiated water (HTO) at the outside of the skin,

was found to be 0.6 mg cm^{-2} h^{-1} (DOWNES et al., 1967), the same value as the water vapor loss from the skin. The barrier simply possesses a resistance to the transport of water molecules and it is irrelevant whether these water molecules are passing from the inside to the outside of the skin or vice versa. It is also irrelevant whether the water is in the liquid or in the gas phase on one or both sides of the skin. The resistance to water loss from normal forearm skin is not governed by the phenomenon of evaporation, but primarily by the resistance or barrier against water transport of the stratum corneum.

III.4 MEASUREMENT OF THE WATER BARRIER OF A FILM

The resistance of a monomolecular layer against evaporation of water has been measured in several ways (SEBBA & BRISCOE, 1940; LANGMUIR & SCHAEFER, 1943; ARCHER & LA MER, 1954; BARNES & LA MER, 1962). These resistances are of the order of some sec/cm and a considerable amount of water vapor is transported across the layer.

The resistance of skin against water vapor loss is in the order of some hundred sec/cm. Much less water is transported through the skin than is transported through monomolecular layers; therefore a sensitive method of measurement such as an electrolytic water analyzer must be used (van GASSELT & VIERHOUT, 1963; SPRUIT & MALTEN, 1966).

The resistance of layers of substances which have an occlusive effect on the skin is of the order of some thousand sec/cm. The specific resistance of such substances is preferably measured by the same apparatus which is used in the measurement of the water vapor loss of the skin itself. Such a measurement can be carried out by applying the electrolytic water analyzer mentioned before.

Table III.B:

Water diffusion coefficients (D) and solubilities (S) of n-hexadecane, and the resistance of a 0.1 mm thick layer of n-hexadecane against evaporation of water from the surface. Nos. 1 - 5 were calculated from data by SCHATZBERG (1965); no. 6 from present results with an electrolytic water analyzer.

no	temp. in °C	n-hexadecane*) D x 10^5 in cm^2/sec	n-hexadecane*) S x 10^6 in $g\ cm^{-3}$	water vapor, maximum concentration in $\mu g\ cm^{-3}$	resistance of 0.1 mm n-hexadecane in sec/cm
1:	25	4.16	41.6	22.8	132
2:	30	4.59	52.1	30.0	126
3:	35	4.95	64.1	39.2	124
4:	40	5.42	79.1	50.6	118
5:	45	5.97	95.5	65.0	114
6:	22.2	-	-	19.2	133.5

*) values published by SCHATZBERG (1965)

The saturated hydrocarbon n-hexadecane, which is commercially available in a highly purified grade because it is applied in the calibration of gas chromatographs, is very useful in the calibration of the electrolytic water analyzer when measuring the resistance of layers of substances against water evaporation. The diffusion of water through n-hexadecane has been studied by SCHATZBERG (1965) at various temperatures and his figures allow the resistance of layers of n-hexadecane against evaporation of water to be calculated. A 0.2 mm layer of n-hexadecane has the same resistance as forearm skin against water vapor loss. This resistance was shown to be only slightly dependent on the temperature (Table III.B). Formulae (1), (2),

(3), and (6) are referred as basic formulae in the calculation.

In the measurement of the evaporation of water through a film of any substance, a layer of 2 to 5 mm is optimal. SCHATZBERG (1965) estimated the decrease of the weight of a beaker, containing some water covered by a film of the investigated substance. The surrounding air was maintained anhydrous with $MgClO_4$. A recording microbalance was used by SCHATZBERG.

In our measurements, a wash bottle was used; the internal diameter and hence the diameter of the film of investigated substance was 15.9 cm^2. Dried air or dry nitrogen is led through a tube into the wash bottle at a height of about 1 cm above the surface of the investigated substance, applying a flow of about 100 ml air per minute. The water vapor passing from the liquid phase through the layer of substance covering the water is carried by the air or nitrogen to the electrolytic water analyzer and estimated by this instrument. An equilibrium state is obtained after some hours. The equilibrium state obtained in a blank measurement is subtracted. The water loss from a 5.2 mm layer of n-hexadecane at the water surface at 22.2 ^{o}C (no. 6 of Table III.B), was found to be 0.80 mg cm^{-2} h^{-1}. From this figure the resistance of a 0.1 mm thick layer of n-hexadecane is calculated to be 133.5 sec/cm; this is in very good accordance with SCHATZBERG's values, especially because the accuracy of the electrolytic water analyzer is stated by the manufacturer to be only 5 %.

White vaseline has been examined by BAKER (1968) as an occlusive ointment base. It is called white vaseline or vaselinum album in the Swiss and Dutch pharmacopoeias, soft white paraffin or paraffinum molle album in the British pharmacopoeia, white petrolatum or petrolatum album in the United States pharmacopoeia. It is an unctuous, transparent material with a melting point between 40 and 50 ^{o}C, widely used in dermatology. It

is derived from a residual fraction in petroleum distillation. The resistance of white vaseline has been measured in the same way as the resistance of n-hexadecane, using a 2.7 mm layer. The resistance of a 0.1 mm thick layer of white vaseline appeared to be 8300 sec/cm at 23 ^{o}C and 6000 sec/cm at 33 ^{o}C, as has been mentioned by SPRUIT during the discussion of BAKER's paper at the Symposium on Skin at Eastbourne (1968).

III.5 INFLUENCE OF A FILM ON THE WATER BARRIER OF THE SKIN

The thickness of a layer of liquid on the skin can easily be determined by weighing the amount of liquid applied to the skin by pencilling. It is about 0.02 mm thick when n-hexadecane is applied until the skin is glittering.

When white vaseline is applied liberally (therapeutically). a 0.3 mm thick layer is obtained. If such a layer is left on for 15 minutes and then scraped off the stretched surface with the flat side of a wooden spatula, it leaves a glittering film on the skin's surface: the thickness of this film is a maximum of 0.05 mm for healthy skin on the forearm. The maximum thickness of the layer has been calculated from the weight of the vaseline applied to 5 x 10 cm^2 skin; it is not possible to calculate the precise thickness because a certain amount of spreading occurs during the 15 minutes interval before the excess is removed.

As an example, some measurements of the water vapor loss of forearm skin obtained before and after the application of white vaseline in this way have been summarized in Table III.C. The layer of vaseline was originally applied over 5 x 10 cm^2 skin, but it had spread over a larger area during the first 15 minutes - perhaps about 8 x 13 (about 100) cm^2 - and the final thickness of the film was probably about 0.03 mm. The resistance

Table III.C:

The water vapor loss of forearm skin in vivo before and after application of white vaseline as described in the text.

	water vapor loss, measured in mg cm^{-2} h^{-1}	resistance against water vapor loss in sec/cm	increase of resistance in sec/cm	relative barrier resistance	
1: before application:	0.60	210	0	1	x
2: at the end of 15 min therapeutical application and scraping off excess vaseline by a wooden spatula:	0.054	2300	2090	11	x
3: 45 minutes afterwards:	0.192	660	450	3.1	x
4: one hour after 3:	0.150	840	630	4.0	x
5: one hour after 4:	0.144	880	670	4.2	x
6: following sweating and one hour after 5:	0.27	470	260	2.2	x

against water vapor loss was increased by the application by 2090 sec/cm. Such a resistance, in fact, corresponds to an 0.035 mm thick layer of white vaseline, as the resistance of the vaseline alone is 600 sec/cm per 0.01 mm.

The vaseline covered skin does not maintain this high resistance for long. The resistance soon decreases irregularly until a value of about 800 sec/cm has been reached and it remains

at this value for some hours. Sweating may decrease the resistance against water vapor loss still more, but the resistance nevertheless remains more than the original value of the naked skin. An occlusion of the skin has been obtained for some days.

Vaseline will spread over the surface of the skin and sweating will facilitate this spreading. It can, therefore, be supposed that the above resistance has decreased because of such spreading phenomena. The resultant resistance of the vaseline covered skin is, however, also influenced by certain other factors, which will be discussed in the next paragraphs.

The surface of the skin is not completely flat (JENKINS & TRESISE, 1968); it contains grooves, even when it is stretched. The film of vaseline covering the skin will, after some time, acquire a variable thickness, which is schematically represented in Figure III.1. An increased thickness at one site at the cost of a decreased thickness at another site decreases the

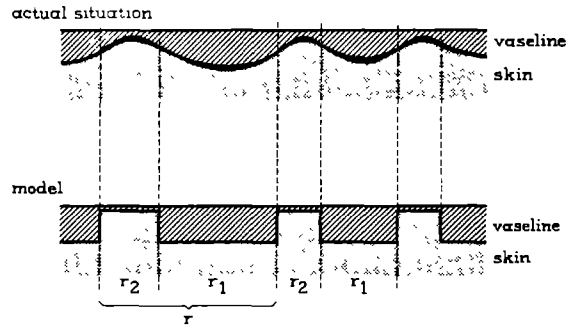


Figure III.1:
The vaseline layer covering the skin surface in measurements 3 *et seq.* (Table III.C) is supposed to have become non-uniform in thickness. Some time after scraping off the excess vaseline, the layer becomes reduced over part of the test area so that the resistance to water transport is essentially that of almost uncovered skin (r_2) while at other parts of the test area the resistance to water transport is increased (r_1), as schematically represented in the model.

total resistance $\underline{r}$. The resultant resistance $\underline{r}$ is dependent on the two parallel resistances r_1 and r_2 as:

$$1/r = 1/r_1 + 1/r_2 \qquad (24)$$

If we simplify in the first instance that a part of the skin, A, is covered by the vaseline, and another part, 1-A, is uncovered, and the thickness of the original vaseline layer following on scraping is known, the resistances r_1, r_2 and $\underline{r}$ can be calculated. It has already been mentioned that this thickness is known very roughly, and therefore it may be represented by the number of 0.01 mm thick layers of vaseline, $\underline{n}$, originally present. The resistance of one layer of 0.01 mm vaseline to water transport is 600 sec/cm. The resistance of a vaseline layer of 0.01 $\underline{n}$ mm thick is therefore 600 $\underline{n}$ sec/cm. The resistance of human forearm skin, mentioned in Table III.C, is 210 sec/cm. On the basis of the measurements of Table III.C follows as an example:

$$r_1 = \frac{210}{A} + \frac{600\ n}{A^2} \quad \text{sec/cm} \qquad (25)$$

and:

$$r_2 = \frac{210}{(1-A)} \quad \text{sec/cm} \qquad (26)$$

Formulae (24), (25), and (26) result in:

$$r = \frac{210\ A + 600\ n}{A - 2.86\ n\ A + 2.86\ n} \quad \text{sec/cm} \qquad (27)$$

The resistances of originally 0.03 mm and 0.06 mm thick layers of vaseline have been calculated for various values of A (the fraction of skin covered with vaseline), see Table III.D. According to the results of Table III.C, the resultant resistance of the vaseline covered skin was originally about 2300 sec/cm and

Table III.D:

Comparison of resultant resistances of skin, covered by originally 0.03 and 0.06 mm thick vaseline layers, calculated in connection with data of Table III.C and the model Figure III.1.

(Resistances in sec/cm)

skin fraction 1: (A cm^2) covered by vaseline	skin fraction 2: (1-A cm^2) devoid of vaseline	resistances to water transport of skin, covered by a vaseline layer, being originally: 0.03 mm thick r_1	r_2	r	0.06 mm thick r_1	r_2	r	difference of resistances r rel. to the mean: Δ r in %
0.0	1.0	∞	210	210	∞	210	210	0.0
0.1	0.9	182,100	233	233	362,100	233	233	0.2
0.2	0.8	46,050	262	260	91,050	262	261	0.4
0.3	0.7	20,700	300	296	40,700	300	298	0.7
0.4	0.6	11,775	350	340	23,025	350	345	1.4
0.5	0.5	7,620	420	398	14,820	420	408	2.5
0.6	0.4	5,350	525	478	10,350	525	499	4.3
0.7	0.3	3,973	700	595	7.646	700	641	7.4
0.8	0.2	3,074	1050	783	5.887	1050	891	12.9
0.9	0.1	2,457	2100	1132	4,677	2100	1449	24.6
1.0	0.0	2,010	∞	2010	3,810	∞	3810	61.8

fell to about 800 sec/cm. According to Table III.D this final figure therefore suggests that 80 % of the skin remains covered by vaseline and 20 % is devoid of vaseline according to the model of Figure III.1. It is not important whether the layer of vaseline was originally 0.03 mm or 0.06 mm thick; the difference

between the resultant resistances is only about 13 %.

The simplifying assumption of the model of Figure III. 1, that in scraping off excess vaseline from the skin, the layer of vaseline is so reduced over some parts of the test area that no vaseline is left at this site, is certainly not true, as the skin is left glittering after the scraping. At site no. 2 the thickness of the vaseline layer will not be reduced to zero but to some low value h. The resistance at that part of the skin is not that of the uncovered skin (r_2 of formula 23 and Table III.D), but to an intermediate value, which can be calculated similarly. The results of such calculations, assuming that h is 0.0003 mm, 0.003 mm, 0.01 mm, 0.02 mm and the original 0.03 mm (that is 1 %, 10 %, 33 %, 67 %, and 100 % of the original thickness of the layer, respectively), are represented in Figure III.2. Evidently the presence of a thin film of vaseline (e.g. about 1 % of the original thickness) does not appreciably change the results shown in Table III.D and Figure III.2. Even if 40 % of the skin surface is assumed to be covered by a 0.003 mm thick layer of vaseline (10 %) and 60 % by a thicker layer, the resultant resistance of the vaseline covered skin is still reduced to about 800 sec/cm. These calculations make it plausible that the factor of non-uniform thickness of the vaseline layer on the skin has caused the decrease in resistance to water vapor loss to some 800 sec/cm.

The thickness of the horny layer of the skin of the forearm is about 0.02 - 0.03 mm. It seems improbable that the mean thickness of the vaseline layer at sites no. 1 of the skin will be increased by more than 0.03 mm. These not very probable situations are covered by the darkened area of Figure III.2.

Sweat gland duct openings will probably soon lose most of the vaseline film, as this film will be removed by the sweating process. It must also be remembered that sebum ducts terminate near sweat gland duct openings and that the vaseline

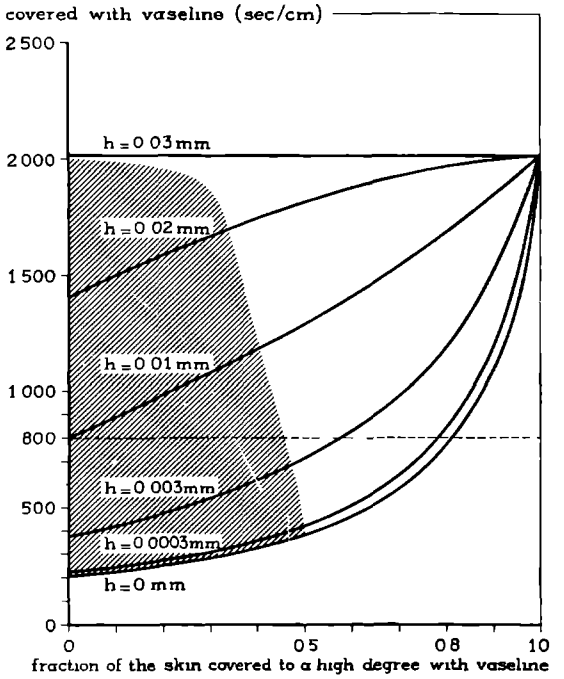


Figure III.2:

Calculated resistance to water transport of forearm skin covered with vaseline (example from Table III.C) in the case of a non-uniform vaseline film. The curve h = 0 mm corresponds to the values of column 4 of Table III.D with thickness 0.03 mm. The thickness indicated by h is that of the thin part of the vaseline layer (see Figure III.1). The shaded area indicates the situations in which the thickest part of the vaseline layer on the skin has been increased by more than 0.03 mm.

will soon be mixed with sebum and in this way become less resistant to water transport. The question arises as to whether such processes at the sweat duct openings may contribute to the decrease of the resistance of the skin to water transport. Sweat gland duct openings take up a space of about 1 % of the surface area of the forearm skin, so that a fraction A = 0.99 remains covered with a thick film of vaseline and only a fraction 0.01 may be devoid of vaseline. According to Figure III.2, the influ-

ence of such small fractions appears to be of minor importance, even supposing the complete removal of vaseline from the sweat duct openings (h = 0).

The vaseline will be mixed with other substances of the skin surface and its specific resistance will be decreased. SCHATZBERG (1965) remarks that an addition of unsaturated groups to a saturated hydrocarbon causes an increased solubility of water in the material. Because of this increased solubility coefficient the permeability coefficient will also be increased, and the resistance against water transport decreased. ARCHER & LA MER (1954) found the specific resistance of a monomolecular layer of pure nonadecanoic acid decreased to 83 % of its original value by inclusion of 0.01 % foreign molecules and decreased to only 5 % of its original value by inclusion of 1 % foreign molecules.

From a dermatologists' viewpoint, it is only important to know the result of the application attained, which will vary for each individual skin. The term "occlusivity" has been proposed by BAKER (1968) and was only evaluated by him in terms of "partial occlusivity" and "complete occlusivity". Quantitatively this occlusivity can be evaluated as the "relative barrier resis- "tance" as has been done in Table III.C, last column. A "partial "occlusivity" corresponds with a "2 x" and a "complete occlusi- "vity" corresponds with a "4 x" relative barrier resistance.

III.6 SOME CHARACTERISTICS OF THE EPIDERMAL WATER BARRIER

The human skin is a very large organ. Its detailed composition and function are not the same all over the body. Important differences exist, for example, between the skin of the forearm and the skin of the palm of the hand, the latter being about ten times as thick as the former, having about ten times as many

sweat glands per unit area and an insensible water vapor loss about seven times as high (BURCH & WINSOR, 1946; KUNO, 1956; SPRUIT & MALTEN, 1965; see also: van KOOTEN & MALI, 1966; SCHEUPLEIN, 1966). There are, however, also distinct differences in water vapor loss which cannot be related to structural or histologically visible differences. Although histologically no difference can be seen between the skin of the scrotum and the skin of the abdomen (equally thick stratum corneum and similarly structured, as seen by haematoxylin-eosin staining), there are marked differences in water permeation. The water vapor loss of scrotal skin is 2 - 20 times as high as the water vapor loss of abdominal skin (SMITH et al., 1961).

A layer of lipids is present around each keratin fibre of the stratum corneum (SWANBECK & THYRESSON, 1962). The character and the amount of these lipids influences the water vapor loss (MALI, 1956; ONKEN & MEYER, 1963; BLANK et al., 1967). They can be altered for various reasons; some of them may be mentioned. The chemical saturation of lipids can vary when the temperature of the environment is changed, causing a change of the solubility and/or of the phase (RING, 1965). The phase can also be changed from oil-in-water into water-in-oil by an addition of Ca^{++}-ions, as for example is observed in plasma (CERBÓN, 1965; HAGLUND & LØVTRUP, 1966).

Biological membranes are thought to be composed of one or more monomolecular layers, usually bimolecular layers. The resistance against water transport of a continuous synthetic phospholipid bimolecular layer (about 50 Å thick) has been estimated by VREEMAN (1966) as being 0.005 sec/cm, so that a 0.2 mm thick layer at 33 °C will have a resistance of about 200 sec / cm. The horny layer of the forearm skin has about the same resistance against water transport, but is only about 0.02 mm thick. According to one of the proposed models of such biological bimolecular layers, "statistical" pores are present, through which

pores material can be transported. KOEFOED-JOHNSEN & USSING (1953) have remarked that an average diameter of the pore of 20 Å is sufficiently large to let water pass in clusters of water molecules, as it moves in water itself (self-diffusion). The energy for activation of the water permeation can be reduced by widening the pores or "channels". HAYS & LEAF (1962) widened the pores of frog skin membrane hormonally and found a 5.7 Kcal/mole reduction in the activation energy. SCHEUPLEIN (1966) measured the permeation of water through human skin (P) at various temperatures and found the following relation:

$$P = 10.5\ e^{-6.0/RT} + 10^{11}\ e^{-19.7/RT} \qquad (28)$$

This indicates that there are two individual energies of activation for permeation (6.0 and 19.7 Kcal/mole). He concluded that two different pathways are located in parallel in the human skin. Pore diffusion - through partial openings such as intercellular spaces and appendages which occupy a small fraction ($f = 10^{-5}$) of the stratum corneum membrane - is dominant at low temperatures, but increases only slowly with temperature owing to the small activation energy of 6.0 Kcal/mole. Bulk diffusion - to be interpreted as water transport through the "immobilized" water contained within the membrane itself - rapidly becomes dominant at higher temperatures owing to the larger temperature coefficient of 19.7 Kcal/mole. At room temperatures the flux via both mechanisms is approximately equal, according to SCHEUPLEIN (1966). He mentions that hydration of the keratin filaments will influence the phenomena.

In general the epidermal cells can be classified into at least three major groups: a) the cells producing the "soft" keratin of the stratum corneum, b) the cells producing the "hard" keratin of the hair, c) the cells producing the fatty sebum of the sebaceous glands. In normal situations these cells either undergo

mitosis or produce their typical products, but in abnormal situations they are found to be interchangeable. After destruction of the skin surface any of these epidermal cells may contribute cells towards the formation of a new and entirely typical surface epidermis. It can, therefore, be doubted whether the quality of the product remains optimal after an emergency regeneration (SPRUIT & MALTEN, 1965, 1966, 1968; SPRUIT & GOVAERT, 1968). A direct relation of the skin permeability to the mitotic rate has not been demonstrated (SPRUIT, 1965). However, the epidermal chalone which regulates the mitotic rate, is water soluble - and its more active adrenaline complex too - and it can easily be extracted when the epidermis is macerated (BULLOUGH, 1965). The rate of chalone production within the epidermis, its diffusion through the epidermis and its ultimate degradation or elimination normally exist in a steady state of balance which is easily disturbed by removal of the chalone by water extraction. Extraction of the skin with water, other solvents, or the removal of the horny layer, will therefore automatically result in an increased mitosis, and this will occur especially in the summer when the skin is macerated by sweat. Moreover, the extraction of water-soluble substances from the skin increases its water permeability by changing its structural characteristics (water content) (HEERD & OPPERMANN, 1966; JACOBI, 1967; LADEN & SPITZER, 1967). It could reasonably be supposed therefore, that insufficient protective functioning of the skin will be correlated with an increased mitosis, although the reverse of this thesis is not necessarily true.

CHAPTER IV

INFLUENCE OF THE HUMIDITY OF AIR

Summary

The water vapor loss of human skin decreases as the environmental air becomes more humid. This decrease is less than could be expected from FICK's law of diffusion through membranes, because the skin's permeability increases as the ambient humidity increases. These factors have been followed quantitatively by measurements on a piece of cadaverous human skin of the back. The results are represented graphically; they showed that the permeability of skin in vitro was 10 - 20 % higher in ambient humid air than in dry air. The influence of the variations of temperature and relative humidity likely to be encountered in the temperate climate of the Netherlands are considered.

An in vivo investigation was also carried out. The permeability of the skin of the forearm was measured in both ambient humid and dry air, at frequent intervals during a 2 weeks period. The water permeability in environmental humid air was again shown to be about 10 - 20 % higher than the permeability in dry air.

When the skin is sweating and shortly afterwards, the humidity of the micro-climate near the skin surface is increased as compared to the non-sweating steady-state situation. Following sweating, about half an hour is needed to re-

Some parts of this chapter have been published in Dermatologica (Basel) 138: 292-294 and 418-426 (1969).

gain equilibrium. This phenomenon has been demonstrated using the thermal conductivity cell. The amount of water lost during this process has been calculated; it appears that the skin can store 50 - 100 μg water per cm^2 in this way.

IV.1 ABSORPTION AND DESORPTION OF WATER FROM THE HORNY LAYER

The measurement of the water vapor loss of the skin has always been accompanied by many precautionary measures, such as equilibrating for at least one hour, measuring at a fixed time of the day and at a fixed, comfortable temperature of the room, avoidance of previous exposure to water, avoidance of previous sweating and avoidance of previous strenuous physical activities, etc.. Such precautionary measures reduce the scatter of the measurements of water vapor loss, but do not eliminate it (SPRUIT & MALTEN, 1965). As it is now possible to take an almost instantaneous measurement at any degree of humidity of the surrounding air (SPRUIT, 1967; this dissertation, Chapter II), the question arises as to what degree the water permeability of the skin is influenced by the water content of its horny layer, and by the ambient circumstances influencing the water content of the horny layer. Variations of the water content of the skin's water barrier layer, the horny layer, correlate to some extent with variations of the water permeability. A formula describing the interdependence of the permeability and the water content of a keratin membrane has been given by KING (1945) and has been presented in Chapter I (formula 21). We will not, however, use this formula, especially as the factor $d(A - X)/dA$ may hinder practical application and interpretation, and prefer the graphical way of presentation and interpretation.

The absorption isotherm of the horny layer of the skin of the trunk is shown in Figure IV.1. It is evident from this absorption isotherm that the water content of the horny layer at a fixed humidity of the air, e.g. a % RH, may be p % or q %, depending on the way in which the humidity was reached. If the humidity of a % RH has been reached by an increase of a lower humidity of the air, the value of q % water content will be relevant; if the humidity of a % RH has been reached by a decrease of a higher humidity of the air, the value of p % water content will be relevant. Realization of any value of the water content between p % and q % at a % RH of the air is thus possible. Figure IV.1 represents the locus of all possible values of the water content of the horny layer at various degrees of humidity of the air, the locus being not only both lines of the absorption isotherm but also the complete area between these lines. This behavior is similar to hysteresis phenomena.

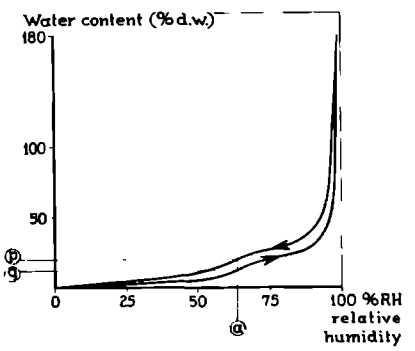


Figure IV.1:
Absorption isotherm for the horny layer of the epidermis of the trunk at 20 °C (see MALI, 1956). At a relative humidity of the air of a % the water content of the horny layer may be between the limits of p % and q %.
(reprinted from Dermatologica 138: 418-426, 1969)

Most synthetic membranes tend to absorb water, and their water permeability has been studied extensively (see MOLL,

1964). The water permeability has been found to vary with the water content of the membrane. As the water content of the membrane is related to the humidity of the surrounding atmosphere according to the hysteresis phenomenon (Figure IV.1), it can be predicted that the water permeability through the membrane will vary with the humidity of the atmosphere in much the same way. It can thus be predicted that the observed value of the water permeability will fluctuate between two extreme values, largely dependent on the way in which the environmental humidity has changed. KING (1945) carried out experiments on 0.05 mm synthetic keratin membrane, which showed that the water content of the membrane and its water permeability were related in accordance with the hysteresis phenomenon. Subsequently a great deal of work has been carried out by other workers (GAUL & UNDERWOOD, 1952; FLESCH & ESODA, 1957; PEISS et al., 1956; HALE et al., 1958; BUETTNER, 1959; BUETTNER & HOLMES, 1959; HOLZMANN et al., 1961; HEERD & OHARA, 1962; CHERNOSKY, 1962; SHELLEY & RAWNSLEY, 1964; HEERD & OPPERMANN, 1966; BETTLEY & GRICE, 1967; JACOBI, 1967; GRICE et al., 1968).

In order to investigate whether a similar relation might exist between the water permeability of human skin and the humidity of the environmental air and to what extent such a relation might influence the actual water vapor loss of the skin, the water vapor loss of cadaverous human skin of the back has been measured at various ambient humidities.

IV.2 WATER PERMEABILITY OF THE SKIN IN VARYING AIR HUMIDITY

In this experiment a piece of cadaverous human skin of

the back was placed upon a metal screen - dermal side downward - and was immersed in a physiologic salt solution at a constant temperature of 30 ^{o}C. A 2 cm^{2} cup was placed upon the epidermal side of the skin (see Figure II.7), and a flow of 10 ml air per minute was passed through the cup. The amount of water taken up by the air from the skin was recorded by a thermal conductivity measuring apparatus (Figure II.5). This method allowed measurements of the water vapor loss to be performed at any humidity of the air, either the environmental humid air or air previously dried by silica gel (see Chapter II).

The humidity of the ambient air, running over the skin, was changed several times and each time a series of determinations of the water vapor loss was carried out. Between changes of the humidity of the air, the skin was exposed to dry air, and the water vapor loss in dry air was determined as a control (see Table IV.A). During the four days of the experiment the water vapor loss using dried air was measured 5 times in total; this value remained constant at 0.50 mg cm^{-2} h^{-1}. The procedure and the results are represented in Table IV.A and also by Figure IV.2.

According to FICK's law of diffusion it is evident that no water can evaporate into saturated air. Water will evaporate quite easily into completely dry air, however; in such circumstances the skin membrane will lose water vapor rapidly. In our piece of cadaverous skin 0.50 mg water evaporated per cm^{2} skin and per hour into nearly dry air (1.5 % relative humidity). If the skin were an ideal membrane, and if its water content and hence its permeability did not change in varying ambient humidities, all values of the water vapor loss of the skin between extremely dry and completely saturated air humidities would be found upon the skew straight line of Figure IV.2 between the origin and the point (0.50 mg cm^{-2} h^{-1}, 0 % RH). This would indicate that the permeability, as a characteristic of this skin membrane, is 0.50

number of measurement	calibration period before measurement in hours	humidity of the air in % RH	water vapor loss in mg cm^{-2} h^{-1}	variation coefficient of measurement in %
dry	20	1.5	0.50	0.3
1	2	33	0.39	2.5
dry	1	1.5	0.50	1.9
2	1	28	0.43	3.7
3	3	49	0.38	5.3
dry	1	1.5	0.50	2.8
4	0.5	50	0.23	8.7*)
dry	15	1.5	0.50	- *)
5	3	28	0.42	7.4
6	2	34	0.44	5.8
7	0.5	33	0.43	6.0
8	0.5	32	0.44	7.7
9	15	26	0.44	1.9
dry	1	1.5	0.50	4.0
10	1	45	0.38	8.9
11	1	53	0.41	2.8
12	1	56	0.35	8.9
13	1	59	0.39	6.1
dry	1	1.5	0.50	10.5*)

Table IV.A:
Water vapor loss of a piece of cadaverous human skin of the back, at various degrees of air humidity (at 30 °C).

Measurements are tabulated chronologically.
The permeability of this piece of skin was the same from beginning to end of the experiment, namely: 0.50 mg cm^{-2} h^{-1} in dry air.

*) only three measurements were carried out.

mg cm^{-2} h^{-1}. In reality the skin does not behave as an ideal membrane: it absorbs and desorbs water vapor (Figure IV.1). Indeed the measurements of the skin's water vapor loss were not found

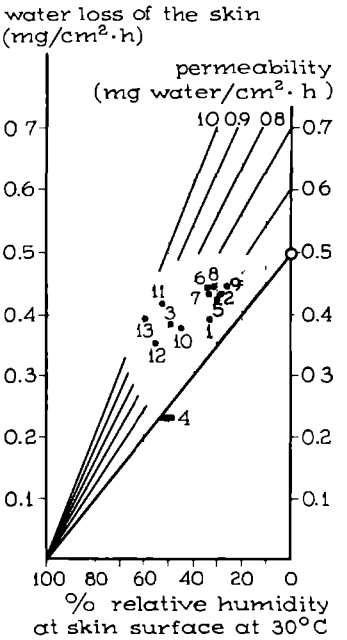


Figure IV.2:

Results of a series of measurements of the water vapor loss from cadaverous human skin of the back, passing air of various degrees of humidity through the measuring cup at 30 °C skin temperature. Other circumstances are as described in the text. Permeability lines at 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mg water cm^{-2} h^{-1} have been included.

(reprinted from Dermatologica 138: 418-426, 1969)

around this unbroken straight line. The decrease in the water vapor loss was less than that calculated for an ideal membrane for all measurements, with no. 4 as the only exception. Evidently, the permeability of the skin in humid air was increased as compared with the permeability in dry air. The value of the permeability of the skin at any humidity of the air can be read from the other skew lines of Figure IV.2. During measurements nos. 1, 2, 5, 9 the skin behaved as having a permeability of 0.60 mg cm^{-2} h^{-1}

in dry air: during measurements nos. 6, 7, 8 the skin behaved as if the permeability were 0.7 mg cm^{-2} h^{-1}; etc..

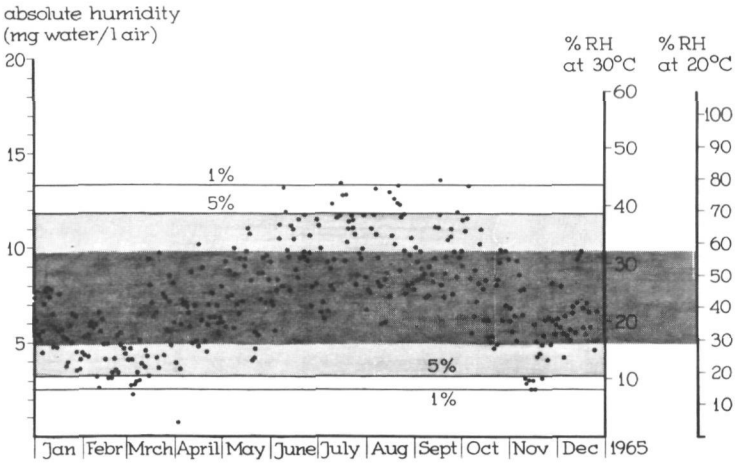


Figure IV.3:
The humidity of the ambient atmosphere at the moment of the maximum temperature of each day on the year 1965 at the Royal Meteorological Institute at De Bilt; absolute humidity is shown of the lefthand ordinate and the relative humidities at 20 °C and 30 °C on the righthand ordinates. The heavily shaded area represents the humidity of sealed cabins and pressure suits, as recommended by WEBB (1965).
(reprinted from Dermatologica 138: 418-426, 1969)

In the Netherlands the relative humidity of the ambient air is very moderate and only seldom increases over 40 % RH as calculated at a typical skin temperature of 30 °C (Figure IV.3). Therefore, the high humidities used in measurements nos. 10, 3, 11, 12, 4 (Figure IV.2) had to be achieved by artificial moistening of the ambient air of the room at an increased temperature. These values indicate more drastic changes of skin permeability in abnormally high humidities. The permeability is even further increased (0.9 mg cm^{-2} h^{-1}) in measurements nos. 11 and 13 at 50 - 60 % RH and 30 °C: it is nearly twice the original value under these conditions.

By way of example, measurement no. 6 may be considered more thoroughly. The humidity was normal, though high; 34 % RH at 30 °C (equalling a humidity of 61 % RH at 20 °C room temperature). The actual water vapor loss in this environmental humid air was 0.44 mg cm^{-2} h^{-1}. This value is about 12 % less than the water vapor loss of 0.50 mg cm^{-2} h^{-1} in dry air. If the membrane had entirely dried out, the measurement at 34 % RH would have yielded an even lower water vapor loss of 0.33 mg cm^{-2} h^{-1}, being about 37 % less than in dry air. Thus the actual water vapor loss decreased only by 1/3 of that theoretically calculated if the permeability were constant. The increase of the permeability due to swelling of the horny layer was about 15 % (from 0.50 to 0.67 mg cm^{-2} h^{-1}). It is clear that the change in water loss, predicted by FICK's law of diffusion, is partially compensated by the accompanying change in the permeability of the membrane.

The actual values of the water vapor loss were found scattered over a wide area of the graph shown in Figure IV.2. This is in complete accordance with the theoretical considerations discussed in Section IV.1, and confirms that the water permeability does indeed change in the same way as the absorbed water content of the horny layer. As far as the Netherlands are concerned, typical ambient conditions are represented by the gray area of Figure IV.2. The humidities recommended in sealed cabins and pressure suits (WEBB, 1965) are also within these limits. Therefore it can be stated that the gray area of Figure IV.2 comprises all the values of the water vapor loss and the permeability which may be expected in a piece of skin whose permeability is 0.50 mg cm^{-2} h^{-1} in dry air.

The locations of measurements nos. 4 and 12 in the graph of Figure IV.2 are somewhat unexpected. The reason for this apparent deviation is not clear. The measurements are, however, not contradictory to the foregoing explanation.

IV.3 LIMITATION OF AGREEMENT OF MEASUREMENTS OF THE WATER VAPOR LOSS FROM HUMAN SKIN IN DRY AIR AND IN ENVIRONMENTAL HUMID AIR

The variablity of measurements of the water vapor loss of skin in vitro into dry air is about 2 % according to the results of the experiments of Table IV.A (namely variation coefficients or relative standard deviations of 0.3 %, 1.9 %, 2.8 %, and 4.0 % in four series of measurements). These results were obtained with the thermal conductivity cell as described in Chapter II. The same variability has been found measuring in vitro with an electrolytic water analyzer method (SPRUIT & MALTEN, 1965). However, a variation coefficient of about 10 % is found in measurements on the skin in vivo (SPRUIT & MALTEN, 1965). The variation coefficient of measurements in vivo is the same when measured on the skin of the palm of the hand, having a very high water vapor loss and many sweat ducts, or on the skin of the forearm, having a moderate water vapor loss and relatively few sweat ducts, in spite of the different water vapor losses measured and in spite of the different structures of these skin areas (see Figure IV.4). Correction of the measurements for the temperature of the skin does not ameliorate the variability of the determinations (SPRUIT, 1966: SPRUIT & HERWEYER, 1967).

Measurements of the water vapor loss of the skin in environmental humid air with its ever changing humidity can be expected to fluctuate even more than measurements in dry air. As an example the average result of nine measurements on forearm skin in vivo and their variation coefficient is presented in Table IV.B, columns "WVL". The average of the three variation coefficients of the measurements in environmental humid air is

Table IV.B:

Mean values of 9 measurements of the water vapor loss (WVL) and the calculated permeability (Perm) in mg cm^{-2} h^{-1} of forearm skin of a normal healthy volunteer in dry air and in environmental humid air. The measurements were carried out during the period from 19th September till 4th October 1966, the absolute humidity varying between 7.9 and 13.4 mg water/liter air.

measurements carried out in	skin site a		skin site b		skin site c	
	WVL	Perm	WVL	Perm	WVL	Perm
1: ambient humid air	0.57	0.84	0.40	0.58	0.35	0.50
2: dry air	0.69	0.69	0.50	0.50	0.47	0.47
Perm 1 - Perm 2 =		= 0.15		= 0.08		= 0.03
idem in %:		18 %		14 %		6 %
	variation coefficients of the above measurements					
1: ambient humid air	8.9%	7.5%	13.0%	7.9%	13.0%	11.7%
2: dry air	9.8%	9.8%	7.0%	7.0%	3.9%	3.9%

11.5 %. The average of the three variation coefficients of the simultaneous measurements in dry air is somewhat less, 7 %.

The water permeability of the skin in environmental humid air and in dry air should have the same value if the skin were structurally unchanged as far as its water content is concerned. It became evident from measurements presented in the foregoing Section IV.2, that the permeability of the skin in vitro changes somewhat (10 - 20 %) because of such structural variations. The permeabilities of the skin in vivo have been calculated from the previous measurements of the water vapor loss. The average values in ambient humid air and in dry air are shown in columns "Perm" of Table IV.B. The permeability of the skin in

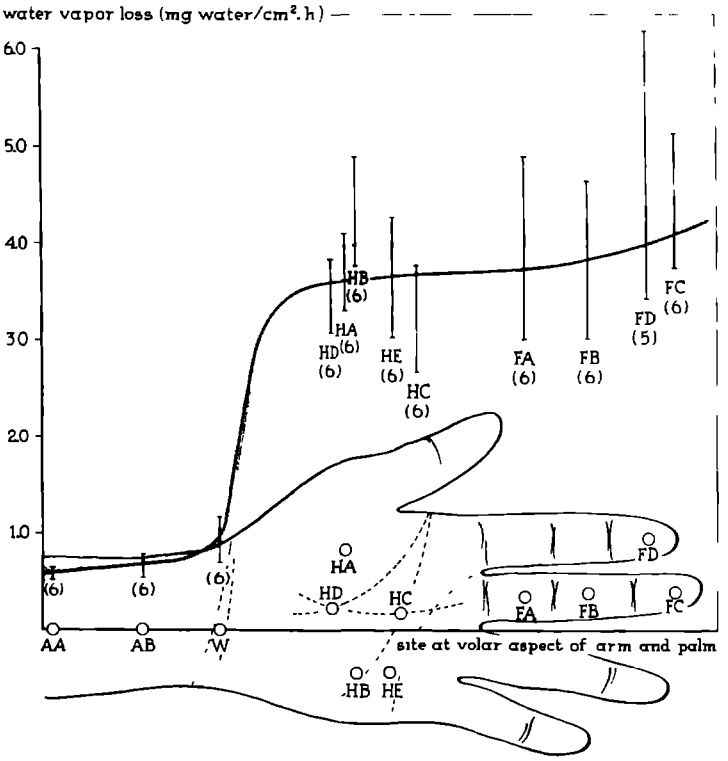


Figure IV.4:
Water vapor loss (insensible perspiration) of the palm of the hand and the forearm skin of a healthy volunteer. Numbers in parentheses indicate the number of determinations of the water vapor loss of different days at the relevant site. The gray area indicates the (relative) standard deviation of the measurements.

environmental humid air is seen to be increased as compared to the permeability in dry air: the magnitude of the increase is about the same as was found in the in vitro measurements (6 to 18 % according to Table IV.B).

Measurements of the permeability of the water through the skin into environmental humid air can be expected to spread less than the measurements of the water vapor loss itself, because the factor of the variability of the changing humidity of the

air is eliminated. The average variation coefficient of these measurements of the permeability was 9 % (see in Table IV.B, columns "Perm"), i.e. somewhat less than the average variation coefficient of the measurements of the water vapor loss itself, this being 11.5 % (see in Table IV.B, columns "WVL"). The value of the variation coefficient of 9 % was not reduced to such a degree that the variability became less than the variability of the measurements into dry air, which amounted to only 7 %. The changing water content of the horny layer influences the spreading of the measurements unfavorably.

Changes in skin permeability in vivo can be expected to be most evident during a wet summer period and for the skin of the palm of the hand. During the month of July. 1966. such conditions were fulfilled in the Netherlands. Ten measurements of the water vapor loss were carried out in this period on the middle of the palm of the hand of one volunteer, both in environmental humid air and in dry air. The temperature of the room was 21 to 23 ^{o}C; the mean temperature of the skin was 34.6 ^{o}C: the humidity varied between 24 and 41 % RH at the temperature of the skin, on an average being 32.7 % RH. The mean water vapor loss from the skin in dry air was 4.17 mg cm^{-2} h^{-1}, when no sudden activity of the sweat glands was evident from the recordings. The mean water vapor loss from the skin in environmental humid air was 3.28 mg cm^{-2} h^{-1}, i.e. only 21 % less. The permeability of the skin in environmental humid air was calculated from these measurements, and was found to be 4.91 mg cm^{-2} h^{-1}, i.e. 18 % more than the measured water vapor loss in dry air of 4.17 mg cm^{-2} h^{-1}. It is seen that the water permeability in environmental humid air of the skin of the palm of the hand is also increased by 10 - 20 % as compared to the permeability in dry air.

IV.4 DECREASING WATER VAPOR LOSS FOLLOWING SWEAT SECRETION

It is commonly supposed that sweat can wet the skin surface. After sweating has stopped, the skin is in equilibrium with a moistened atmosphere, and will only gradually equilibrate to the actual environmental atmosphere. The length of this time lag is important as measurements of the water vapor loss of the skin must not be started before the skin is equilibrated to the actual atmospheric conditions.

There are two factors influencing the final value of the recorded output. One is the lag in response of the instrumentation: this has been described in Chapter II. The other is the slowness of equilibration of the skin itself. Obviously the former must be known before the latter can be evaluated with certainty.

The recording of the water vapor loss in a normal environmental humid atmosphere can, in fact, be completed in a few minutes using the thermal conductivity cell water analyzer, as is shown in curve a of Figure IV.5. The course of this measurement is as follows:

The measuring cup is placed upon the skin about five minutes before the first measurement is to be taken. The metal cup, the thermocouple element inside the cup, etc. (see Figure II.7) have to be equilibrated and this is judged to be accomplished as soon as the reading of the skin temperature is constant. During this period, the air flow is directed according to diagram "zero" of Figure II.5. According to this scheme, the water evaporated from the skin runs through the tube S2 and the righthand flowmeter back into the room without passing the second thermal conductivity cell compartment. At the start of the actual measurement, the air flow is switched to "measure", as in Figure II. 5. Because of the double moistening, which has been described in detail in Chapter II, the recorder registers a small overshoot

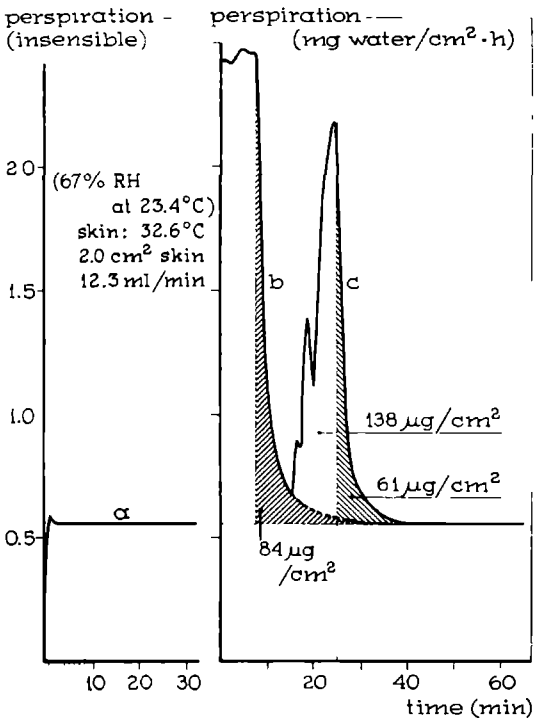


Figure IV.5:

Recording of the water loss of the skin at the volar aspect of the forearm of a healthy volunteer. Measuring cup area: 2.0 cm^2; air flow: 12.3 ml/min; skin temperature: 32.6 °C; room temperature: 23.4 °C; original humidity of the environmental air passing the skin: 14.0 mg water / l air (67 % RH). Curve b-c was recorded during a period of moderate sweating, which ceased; curve a was recorded at the same site of the skin after the sweating had stopped for some time.
(reprinted from Dermatologica 138: 292-294, 1969)

at the beginning of the recording (curve a, Figure IV.5). The value of the insensible perspiration of the skin can be read about 2 minutes after the beginning of the actual measurement. It usually remains constant for a long period, e.g. 15 minutes. It is advi-

sable to check the readings during this period, however, in order to ensure that no sweat gland activity occurs. The effect of sweat gland activity is demonstrated by the measurements of Figure IV.5, curves b-c.

In the beginning of the second experiment of Figure IV. 5 (first 7 minutes of the recording) a substantial water vapor loss of about 2.4 mg cm^{-2} h^{-1} was shown, fluctuating and thereby demonstrating that the sweat glands were moderately active. This limited perspiration caused no visible sweat secretion; a visible sweat gland activity usually results in a water vapor loss about ten times as much. This moderate activity of the sweat glands resulted in an increased perspiration of about (2.4 - 0.6) = 2 mg cm^{-2} h^{-1}. As soon as their activity ceased, the water loss from the skin decreased rapidly to the steady state level of the insensible perspiration (curve b of Figure IV.5). A recurrence of the activity of the sweat glands was observed during the decreasing perspiration period, the result being an increase of the water loss of 138 µg cm^{-2} during a 10 minutes period (see Figure IV.5). Only a quarter of an hour after the sweating had stopped for the second time (at the end of curve c), a constant water loss - the insensible perspiration - was reached. Apparently an excess amount of water slowly evaporated from the skin after the sweat gland activity outbursts ceased. This amount of water is indicated by the area of the graph underneath the descending curves b and c and has been calculated to be 84 and 61 µg water/cm^2 skin respectively. This excess amount of water may have been stored in the horny layer and gradually removed by the stream of environmental humid air, running over its surface through the measuring cup. The percentage of the water content of the horny layer of the skin has been estimated from these data to be decreased from W % in the case of sweating skin to about (W - 4) % at non-sweating skin, equilibrated to the environmental humid air. Whether this surplus amount of water, in

fact, had been stored in the horny cell layer itself or in the sweat ducts cannot be concluded from these data.

Other factors may also influence the evaporation (see KUNO, 1956; EMRICH & ULLRICH, 1966; SLEGERS, 1966).

CHAPTER V

REGENERATION OF HUMAN SKIN

Summary

By repeated stripping of an area of skin with adhesive tape, a progressive injury can be produced. This injury has been used as an experimental model to investigate the relation between the water vapor loss of the skin and the extent of the injury. It has proved particularly useful in clarifying the processes which take place during the period of regeneration.

The water vapor loss has been plotted semi-logarithmically against time following a number of experimental injuries (Figures V.2 and 5). The curves obtained enable three individual situations to be distinguished:

a: Damage to the horny layer above the stratum corneum conjunctum results in a small increase of the water loss returning rapidly but irregularly to normal;

b: Complete removal of the stratum corneum conjunctum causes a considerably greater initial increase; this returns exponentially to normal over a much longer period;

c: Damage which includes the stratum granulosum results in very high values of water loss, and causes a "temporary barrier" to be set up. This is shed after a few days; the regeneration then follows course b above.

The more important parts of this chapter have been published elsewhere; J. invest. Derm. 45: 6-14 (1965) and Berufsdermatosen 16: 11-24 (1968). Courtesy of the publishers.

V.1 INTRODUCTION: ASPECTS OF THE PHENOMENON "DRY SKIN"

The correct functioning of the skin is very easily disturbed. Most persons pay little attention to the affliction of a "dry skin". Yet: "Dry skin is probably the only affliction, aside "from the common cold, which touches everyone . . . at least "once a year." (DAY, 1966). In the Netherlands, it is common in the months of February, March and April, just after mid-winter. Patients having a dry skin complain of rough patches of dry skin, chapping lips, or hands that are not soft. Of course, the condition of their skin had already suffered appreciably before such complaints became evident. The outer skin layer (the stratum corneum) is composed primarily of keratin, an un-nucleated hygroscopic sheath which should contain about 10 % water in order to remain well-conditioned, soft and flexible. When its water content falls below this concentration, the keratin becomes progressively less flexible, rough, brittle, and finally even cracked (BLANK, 1952). The last condition is very well recognized as "dry skin" by the public; however, it has gradually developed and it is very difficult to state when the process had started.

Several environmental factors promote the phenomenon of a dry skin. The greatest offender certainly is a low absolute humidity, occurring in periods of cold weather. A high skin temperature and a good ventilation along the skin surface are, in addition, particularly liable to provoke the phenomenon. An example of such circumstances is the skier who submits himself to considerable air currents in a cold and dry atmosphere, when he whizzes down the slopes.

There are other potential sources of dry skin, such as occupational hazards. Examples are persons who constantly expose their hands and forearms to solvents (painters, service station attendants), and housewives who have their hands quite

often in dishwater or washwater containing household detergents (SUSKIND, 1962).

Whatever the cause of dry skin, environmental or occupational, the skin becomes harsh, rough, red, and cracked. Skin which is cracked can never be rejoined; it can only be made soft and flexible again. This can be accomplished by coating the skin with a material which has a lower permeability than the natural sebum, so that the keratin will have a chance to hydrate again. STEIGLEDER (1962) demonstrated indirectly that vaseline is probably the most efficient of the readily available materials for this purpose. The high "occlusivity" of a film of vaseline applied to the skin has been shown by BAKER (1968). It has also been demonstrated in the present work (Chapter III) that vaseline can add effectively to the water barrier properties of human skin.

After the skin has been injured, it has to recover from this injury. If the skin was in very good condition before the injury occurred, and if the outward environmental circumstances are favorable, e.g. the humidity of the atmosphere is reasonably high, the skin will revert rapidly to its original state. If, however, the condition of the skin is not so good, or if the ambient circumstances are unfavorable, e.g. the humidity of the atmosphere is low, it is possible that the skin will not function properly for a prolonged period. Such an affliction appears as a chapping of the regenerating skin; a dry-skin phenomenon often develops. It has been observed and studied, together with the regeneration of the skin following a standardized injury (SPRUIT & MALTEN, 1965; 1968; this Chapter).

V.2 THE APPEARANCE OF THE SKIN DURING REGENERATION AFTER STRIPPING THE HORNY LAYER

A film of adhesive sellotape (TESA-film no. 4101, 25

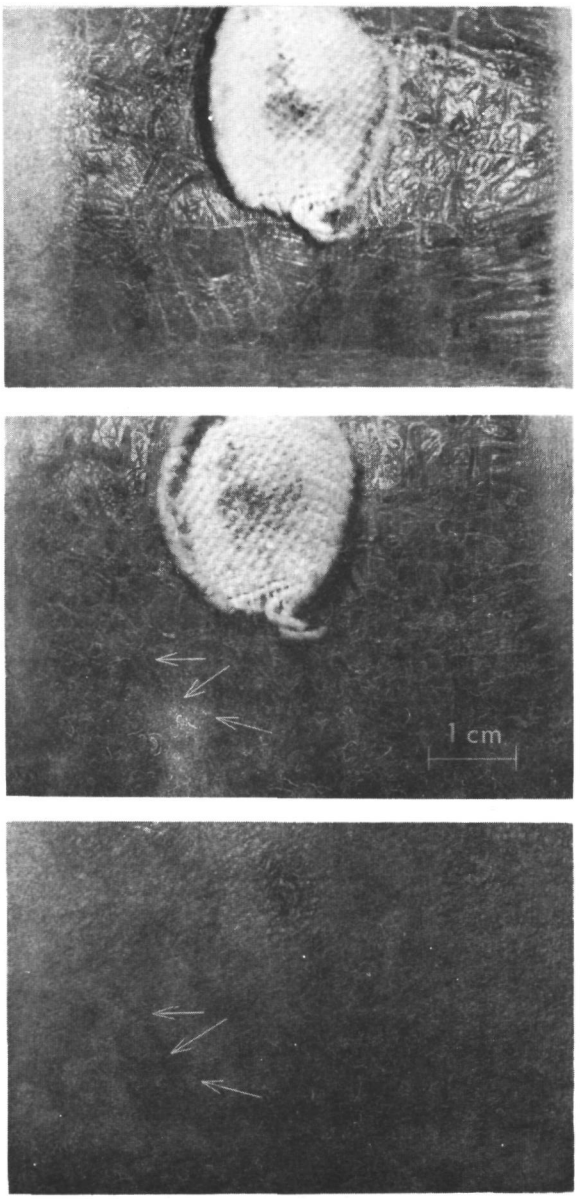


Figure V.1:
Appearance of forearm skin after stripping the horny layer in January, 1963 (mid-winter). The pigmentation pattern, 29 days after injury, corresponds to the chapping pattern 4 and 6 days after injuring the skin (see SPRUIT, 1965).

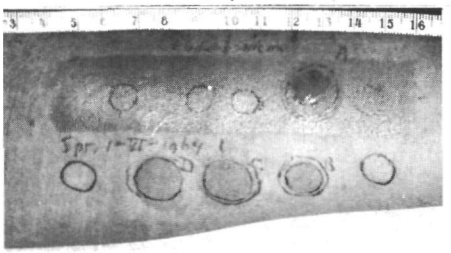
mm width) applied to human forearm skin adheres firmly to the skin. After one or two minutes it is removed by tearing the tape from the skin, and the outmost layer of horny cells is removed together with the sellotape. The same procedure may be repeated several times, successively removing the following layers of horny cells. In this way the complete horny cell layer or stratum corneum, about 0.02 mm thick, can be easily removed from the skin. A part of the underlying, one cell layer thick stratum granulosum is usually also removed by this procedure; the other layers of the epidermis and the dermis are left intact. This procedure is very useful as a standardized method of removing the horny layer from the skin (SZAKALL, 1957).

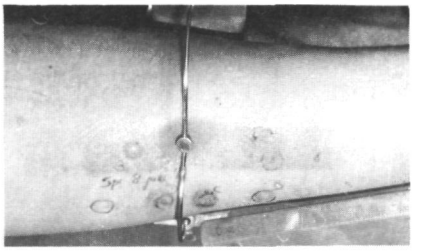
In Figure V.1, photographs a and b, the well-known appearance of forearm skin is shown, 4 and 6 days after stripping the horny layer of the skin in mid-winter; this is a bad time for injuring the skin, as it will easily chap and crack during the following regeneration. Twenty nine days after the stripping (Figure V.1, photograph c) it had regained its flexibility and the injured site is only visible because of a residual pigmentation pattern.

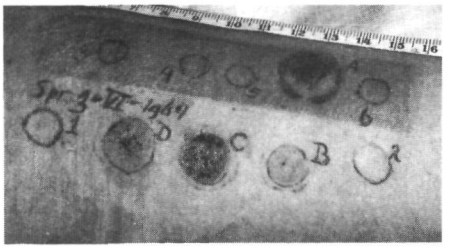
The same skin has also been stripped in the month of June, the climate being very mild at that time. The results are shown in Figure V.2. The injuring effects after stripping (sites 3, 4, 5, 6) are not so bad as in midwinter; however, the newly formed skin still has the characteristics of a "dry skin". Especially during the first regeneration phase the skin is not at all flexible (Figure V.2, photograph c). This layer which is formed during the first few days very effectively protects the body from desiccation, as is evident from the graph of this Figure V.2. Although it is inflexible and lacks smoothness, this layer does not become really rough nor brittle in the summer period. It does not crack but it is gradually lost in flakes (Figure V.2, photograph g). This occurs during the second phase of the regenera-

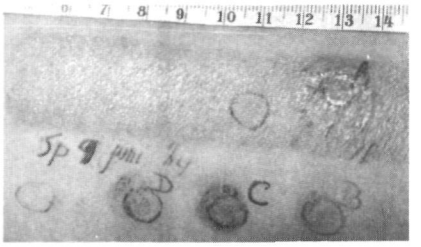
first phase

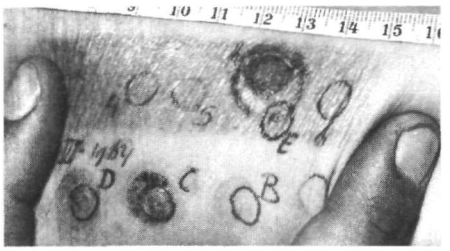
second phase

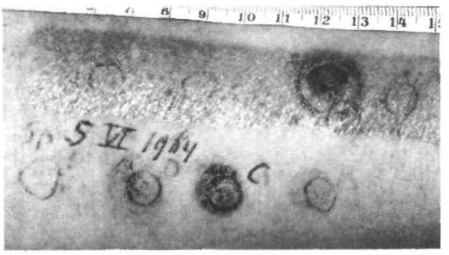


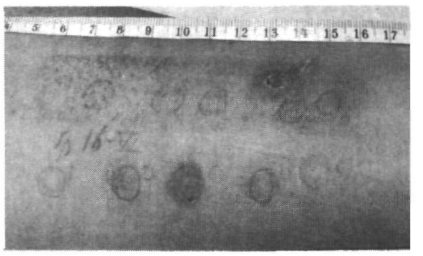












third phase

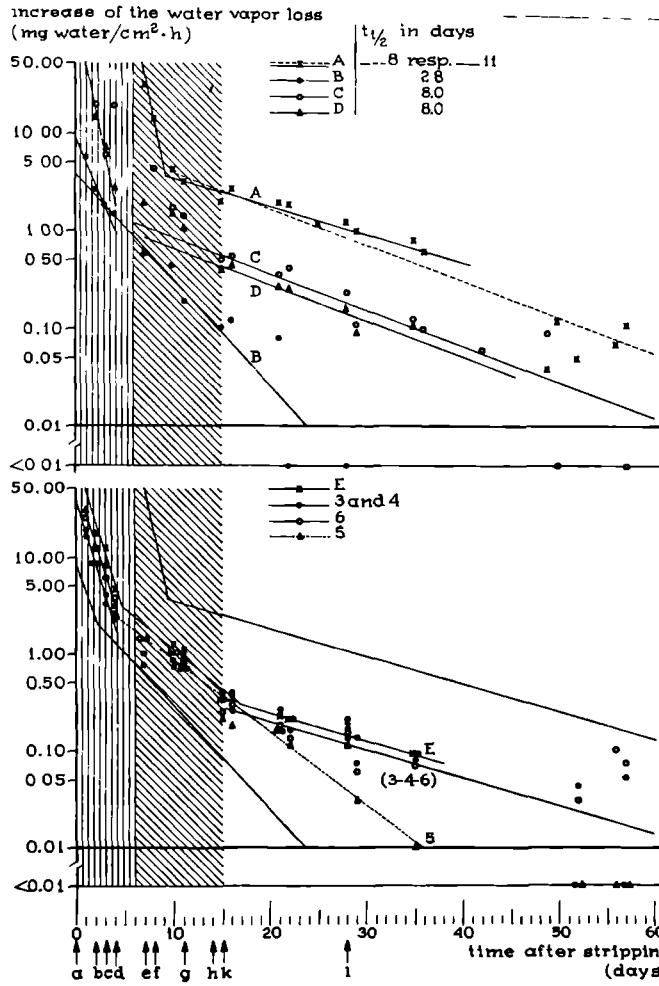


Figure V.2:

The appearance of forearm skin during regeneration after removal of the horny layer by stripping and after injury to the skin by exposure to alkali. The increase of the water vapor loss at each site of the skin during regeneration is shown in the graphs. Sites nos. 1 and 2 are the controls of normal non-injured skin. The horny layer of the skin has been stripped at sites 3, 4, 5, 6, A, and E. At site E, a plastic cup has been fixed to the stripped skin by nobecutane during the exposure to 0.02 N NaOH at site A. At sites B, C, and D the skin has been exposed to 0.02, 0.03 and 0.03 N alkali. Three phases are distinguished: a) 0 - 5 days after injury (temporary barrier formation); b) 5 - 15 days after injury (visibly chapping final barrier formation); c) 15 - 60 days after injury (final barrier formation). (see also SPRUIT, 1965; and SPRUIT & MALTEN, 1966)

tion period (slightly darkened area of the graphs) between 5 and 15 days after the injury; the skin which is formed underneath is more flexible. From the long-lasting increase in pigmentation (Figure V.2, photograph 1) it is evident that this skin is not completely normal. This is confirmed by the water vapor loss of the skin, which only very gradually returns to normal values (Figure V.2, graphs).

When the skin was injured in another way, e.g. by exposing it to alkaline solutions of moderate strength (0.02 - 0.03 N NaOH: Figure V.2, sites B, C, and D), neither the appearance nor the increase of the water vapor loss were very much different from the standardized stripping injury. When 0.02 N NaOH was applied to stripped skin, however, (Figure V.2, site A), the injury was more severe. This more severe injury was clearly visible, as a crust was formed (Figure V.2, photograph b) which shrank (photographs c and d) and was lost 7 days after the skin was injured (photograph e). The skin underneath the crust behaved just as stripped skin did during regeneration.

Care should be taken over stripped skin, immediately and shortly after the stripping injury, as the regeneration of the skin is otherwise influenced somewhat unfavorably. As an example the very slight pressure exerted by the rim of a plastic cup (Figure V.2, site A) caused the occurrence of a crust and ultimately increased the injury to such an extent that it was still visible as pigmentation 28 days after the injury took place (Figure V.2, photograph 1). The cup had been fixed to the skin by nobecutane, and some of the nobecutane had spread over the stripped skin surface inside the cup. It became evident from the appearance of the skin at this site during the regeneration, and from the water vapor loss measurements during this period of regeneration, that the presence of nobecutane itself did not harm the stripped skin nor influence the process of the regeneration. The presence of a film of nobecutane over the stripped skin surface,

moreover, appeared to protect the stripped skin from the injuring effects of exposure to 0.02 N NaOH.

Following the removal of or injury to the horny layer, a new horny cell layer is formed by a rapid conversion of granular cells. This is composed of parakeratotic, nucleated cells instead of normally keratotic un-nucleated cells (MATOLTSY et al., 1962). Because these parakeratotic nucleated cells are evidently different from the normal horny cells and because they are afterwards shed visibly in flakes, this parakeratotic layer (which persists for about five days) has been called a "temporary" barrier by MATOLTSY et al. (1962).

The rapid formation of this "temporary" barrier results in the sweat ducts being improperly constructed through the entire layer. It is a day, perhaps a few days, before the duct takes up the spiral form which it has in normal skin. As a result the temporary barrier effectively blocks the sweat duct (SPRUIT & GOVAERT, 1968). In this respect the temporary barrier resembles the parakeratotic psoriatic horny layer (JUHLIN, 1967). The sweat gland activity is impeded. Wherever the temporary horny layer is broken and cracked, the sweat gland activity is restored within a few days; only where the temporary layer remains unbroken is the sweat gland activity impeded. The temporary barrier produces a miliaria-like reaction if the sweat glands are forcibly actuated by ambient circumstances in the meantime.

The response of the skin to an injury has many aspects. The simple removal of a small part of the horny layer causes an increased glycogen content of the lowest layer of the epidermis, the stratum basale, during the following eight hours. A sequence of events is set up throughout the entire epidermis: the mitotic rate is ultimately increased. This increase of mitoses starts about 30 hours after the removal of horny cells: it reaches a maximum at about 40 hours, and the mitoses are diminishing again

about 50 hours after the removal of horny cells (BROPHY & LOBITZ, 1959; SPRUIT, 1965). It can be expected that such a sequence of reactions will be reflected in the function of the skin, e.g. its protection against water vapor loss.

V.3 THE MODEL OF THE REGENERATION OF THE SKIN FROM A SINGLE INJURY

In order to achieve a better understanding of the regeneration which follows a typical injury to the skin in the complex environmental conditions usually encountered, it is useful to design a model based on a very simple injury. The results of more complex injuries of the skin may then be compared with the model.

As a measure of the protective function of the skin, the water vapor loss (insensible perspiration) has been chosen. This water vapor loss is increased when the skin has been damaged; it will gradually return to normal during the period of the regeneration of the skin.

As a measure of the severity of the injury of the skin (characterized by an index: i) the relative increase of its water vapor loss has been introduced (SPRUIT & MALTEN, 1965):

$$i = (w_r - w_n) / w_n \qquad (29)$$

w_r = the water vapor loss of the regenerating skin,
w_n = the water vapor loss of normal non-injured skin.

After skin has been injured it will start to regenerate. There may be a lag time in the initiation of this process, but as soon as the regeneration has begun, it is regulated by a feedback mechanism. The regeneration will proceed more gradually in the later stages when the original injury is less severe. It has been shown (SPRUIT & MALTEN, 1965) that a semi-logarithmic rela-

tion exists between the injury-index, $\underline{i}$, and the time after injury. The following formula was presented and has since been applied in many determinations of injury (SPRUIT, 1965; SPRUIT & MALTEN, 1965; 1966; 1968; MALTEN & SPRUIT, 1966; MALTEN et al., 1968; BAKER & KLIGMAN, 1967):

$$\log (w_r - w_n) = k_1 - k_2 \cdot t \qquad (30)$$

$(w_r - w_n)$ = the increase of the water vapor loss (IWVL) of the injured skin over the normal value of the insensible perspiration; k_1 = the constant value of the logarithm of the IWVL immediately after the injury; k_2 = a constant dependent on the rate of regeneration; t = the time passed since the injury was accomplished. The formula has shown its practical usefulness for graphical presentation of the regeneration of skin.

The formula can also be written in another way:

$$t_{\frac{1}{2}} \log \frac{(w_r - w_n)_{t=0}}{(w_r - w_n)_{t=t}} = t \log 2 \qquad (31)$$

$t_{\frac{1}{2}}$ = the so-called "half-regeneration-time", being the period which elapses before the IWVL falls to half its original value. The value of the half regeneration time, $t_{\frac{1}{2}}$, can be calculated from the semi-logarithmic plot, and is inversely proportional to the value of k_2 in any particular experiment.

It is evident from these formulae that the water vapor loss of a normal skin site has to be measured as a control. An actual series of measurements should be preceded by a series of measurements at several sites of the original normal skin in order to determine whether the water vapor losses are constant. Usually a difference of water vapor loss was found to exist between the sites near the wrist at the volar aspect of the forearm and the more proximally situated sites. The same water vapor loss was often found from parallel skin sites as e.g. the sites X,

A and B, the sites Y and C, or the sites Z and D of Figure V.3. At site Z the water vapor loss was increased as compared to sites X and Y (the average being 0.535 as compared with 0.406 and 0.424 mg cm^{-2} h^{-1} respectively) because site Z is approaching the wrist fold and hence the character of the skin of the palm. In the case of a stripping experiment at sites A to D, the sites X, Y and Z were used as controls to determine the water vapor loss of the normal skin at parallel sites of the forearm as compared with injured skin sites. A reliable experiment should apply two controls of nearly the same water vapor loss as sites X and Y. The site Z is less useful as a control and has, in fact, been employed only for a control of site D.

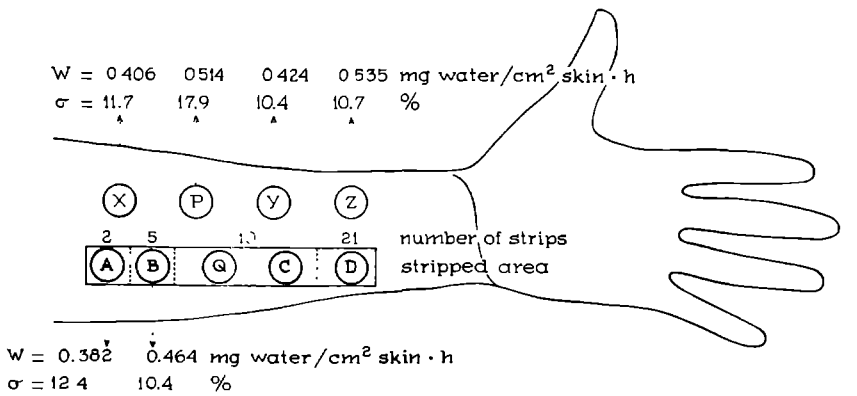


Figure V.3:
The location of the sites of measurements at the volar aspect of the forearm. X, Y, and Z are controls of non-injured skin. The skin at P and Q has been exposed to 0.005 N NaOH. The skin at A-B-Q-C-D has been stripped by sellotape with the indicated number of strippings. At A-B the stratum corneum disjunctum has been only partly removed; the water vapor loss has been slightly increased as indicated.

An appreciable variability between the water vapor loss measurements on different days occurs in normal everyday circumstances in normal skin (see Section IV.3). The water va-

por loss at the control sites is therefore always measured immediately following or preceding the measurement of the water vapor loss at the regenerating injured site of the skin, and the value $(w_r - w_n)$ calculated from the results of these almost simultaneous measurements. The measurement of the water vapor loss w_n at two control sites is also used as a check that no environmental changes occurred during the experimental period, e.g. excessive and longlasting sweating increasing the water vapor loss of the skin. The experimental injury must be the only damage to the skin during the regeneration period, i.e. about a month.

The model used for studying the regeneration of human skin after injury has been derived from experiments as for example the following:

The removal of the horny layer by stripping with adhesive tape was chosen as a reference injury (see Section III.1). The horny layer was removed stepwise (Figure V.3). A part of the skin. site A, was stripped only twice, removing about one third of the stratum corneum disjunctum. Another part of the skin, site B, was stripped 5 times, removing about two thirds of the stratum corneum disjunctum. Sites Q and C were stripped ten times, resulting in a total removal of the stratum corneum conjunctum; the stratum corneum conjunctum started to be removed with the 8th stripping, visible as a compact transparant layer at the sellotape. The remaining site D was stripped 21 times. removing the entire stratum corneum and most of the stratum granulosum. The results of the water vapor loss measurements at these stripped sites of the skin during the regeneration period are presented in Figure V.4.

At site A the water vapor loss was reduced to 0.382 mg cm^{-2} h^{-1} as compared to 0.406 mg $cm^{-2}h^{-1}$ at the control site X. In fact, only 2 out of the 10 measurements of the water vapor loss at site A were found increased above the water vapor loss

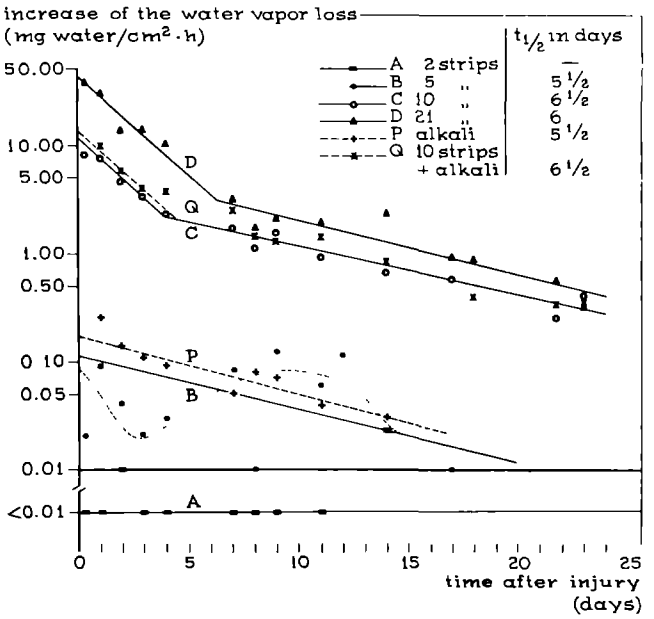


Figure V.4:

The water vapor loss of skin after the injury as indicated in Figure V.3. The entire stratum corneum and a part of the stratum granulosum had been removed at Q-C-D, so that a temporary barrier formation has been set up.

at the control site X and the other 8 measurements of the water vapor loss at site A were found decreased as compared to site X, see Figures V.4 and V.3. There is some indication that removal of only one third of the stratum corneum disjunctum or less will result in an improved protection of the skin to water vapor loss. Theoretically such an effect is not impossible, as the skin responds to even a single stripping by sellotape with an appreciable increase in the number of mitoses about 1.5 day afterwards (BROPHY & LOBITZ, 1959); this will cause a thicker horny layer to be formed than is normally present.

At site B the water vapor loss was increased to 0.464

mg cm^{-2} h^{-1} as compared with the 0.406 mg cm^{-2} h^{-1} at the control site X (Figure V.3). Following removal of about two thirds of the stratum corneum disjunctum at site B, it is seen from graph B of Figure V.4 that all measurements of the water vapor loss were found increased above the water vapor loss at the control site X.

The points of the measurements at site B are certainly not located in a straight line in the graph of Figure V.4. From previous experience it was already known that the regeneration of the skin following the removal of only a part of the stratum corneum disjunctum often does not obey formula (30). Therefore, simultaneously with the stripping of the skin another site, P, was exposed to a very weak alkaline solution, 0.5 ml 0.005 N NaOH per cm^2 skin for one hour. The regeneration of the water barrier of this site P was also followed by daily measurements and comparison with the control sites X and Y. The results are represented in Figure V.4 by the symbol "+". These values appear to be much more linear than the values of the measurements at site B. In other experiments the concentration of the NaOH was reduced even below that used at site P, until ultimately several exposures to water were carried out. Curves similar to the dotted curve B were quite often encountered following these exposures (to be published by MALTEN & SPRUIT, Annali Ital. Derm. clin. sper.); reductions in the water vapor loss, such as were found at site A, did not occur. Slight injuries did not appear to be reproducible using the stripping procedure: they seemed to be more reproducible if produced by other methods.

Summarizing the results obtained by slightly injuring the skin's horny layer, it can be concluded that a slight injury of the skin may increase its water vapor loss by some 0.1 mg cm^{-2} h^{-1}. Measurements of the subsequent water vapor loss often result in a time course as represented by the curved line of Figure V.5; the dashed straight line can also be found. It can be

concluded, that a slight injury or partial removal of the stratum corneum disjunctum (the outer parts of the horny layer) cannot be traced easily by measuring the increase of the water vapor loss of the injured skin.

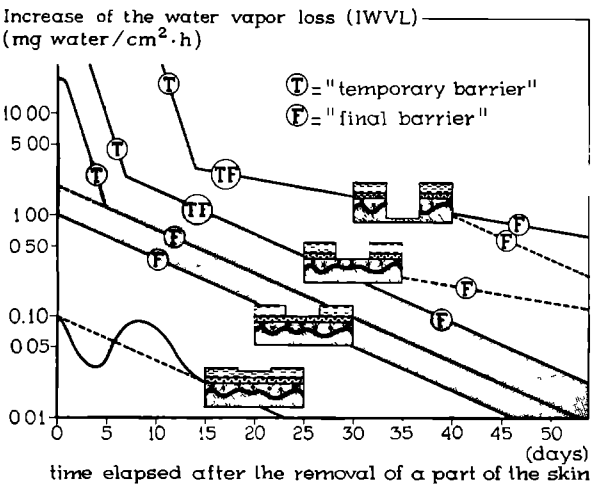


Figure 5.3:
Injury and regeneration of forearm skin following the removal of the illustrated parts of the epidermis: 1: the stratum corneum disjunctum, 2: the entire stratum corneum, disjunctum and conjunctum, 3: the stratum corneum and the stratum granulosum, 4: the entire epidermis. The water vapor loss is very much increased after removal of the entire horny layer, as indicated by the gray area.
(reprinted from Berufsdermatosen 16: 11-24, 1968)

As soon as the stratum corneum disjunctum has been removed by seven strippings of sellotape, the entire stratum corneum conjunctum is removed by the following eighth stripping. Thus the sellotape stripping method is not suitable for removing only a part of the stratum corneum conjunctum. A marked difference is created by the removal of the stratum corneum conjunctum, and this is represented by the difference between the curves

of Figure V.4 corresponding to sites C and B. At site B the stratum corneum disjunctum had not yet been entirely removed and at site C somewhat more than only the stratum corneum conjunctum had been removed; the difference, therefore, between these curves may be somewhat exaggerated. However, a sharp change in the water loss is always found to accompany the removal of the stratum corneum conjunctum.

These changes are represented in Figure V.5. Removal of the entire stratum corneum without removal of any parts of the underlying stratum granulosum usually results in about a 1.0 mg water cm^{-2} h^{-1} increase of the water vapor loss of the skin. The regeneration proceeds very regularly, as is represented by the straight line F. The clinical appearance of this regenerating skin returns almost to normal a short while after the injury, the skin does not show any chapping during the course of the regeneration. A "final" barrier layer begins to form from the very beginning of the regeneration. The function of the skin as a barrier layer against water vapor loss is impaired, however, immediately after the injury; this gradually returns to normal in the course of some weeks (formula 30).

Removal of only a very little more material than just the stratum corneum (i.e. some parts of the stratum granulosum) results in a more drastic increase of the water vapor loss of the skin. In summer and during periods of high ambient humidity, and in a really healthy skin, this increase of the water vapor loss (IWVL) may be restricted to about 2 mg cm^{-2} h^{-1}, and the following regeneration may proceed according to formula (30) in exactly the same way as before, along the continuous line shown in the centre of the shaded area of Figure V.5, beginning at 2 mg cm^{-2} h^{-1}. However, it is usually found that in winter and during periods of low ambient humidity the increase of the water vapor loss (IWVL) is much greater, especially a few hours after the skin was injured. An IWVL of about 20 mg cm^{-2} h^{-1}

can often be found; this was nearly reached at site C following ten strippings (Figure V.4). An additional exposure of such injured skin to 0.5 ml 0.005 N NaOH for 1 hour was carried out simultaneously at site Q (see Figure V.3). This did not appreciably aggravate the injury, as is seen by comparing the relevant curve of Figure V.4 to the curve of the measurements at site C. Only the additional exposure to a somewhat stronger alkaline solution of 0.03 N NaOH causes more severe injury (SPRUIT & MALTEN, 1966).

The IWVL found shortly after an injury is reproducible for any particular specimen of skin, though it can differ immensely between different individuals and is especially dependent on the ambient atmospheric circumstances (summer - winter). Thus values found for the same person during different periods of the year are usually inconsistent.

In the case of the more severe injuries, a temporary barrier is formed (T) which rapidly restricts the water vapor loss during the following regeneration (see Section V.2). Such a parakeratotic temporary barrier is always formed after removal of most or all of the stratum granulosum. This initially results in the complete loss of the water barrier function of the skin (see Section III.2). The temporary barrier formation very quickly (4 to 5 days) reduces the water vapor loss to smaller proportions, as can be seen from the sharp change in the slopes of the graphs between "T" and "TF" (Figure V.5). However, this temporary barrier layer soon becomes dry and brittle. The skin then shows chapping and the temporary barrier layer is shed off. Underneath, a "final" barrier formation has already started. as may be concluded from the following regeneration curve, proceeding with the same slope as the regeneration curves observed after minor injuries. Sometimes, this final barrier continues its good qualities. sometimes it does not. It may dry out and start chapping again after some weeks (lower "TF" -

curve), followed by a real final barrier formation (dashed curve "F"). In this case its regeneration proceeds more slowly than normal.

Following on removal of the entire epidermis by excision - and usually also a superficial part of the dermis - a crust is formed. By very heavy stripping with sellotape some exudate may dry at the skin surface and also cause a slight crust-formation; however, the temporary barrier will start to form immediately after the stripping is carried out, as is evident from curve D of Figure V.4. When the entire epidermis is removed, this protection by temporary barrier formation is postponed, as is shown in the relevant curve of Figure V.5 (see also measurements of SPRUIT & MALTEN,1966). If a crust forms at the beginning of the regeneration period, an exact determination of the IWVL is usually impossible, as leakage of air between the rim of the measuring cup and the crust tends to occur. In a few cases, however, such a determination has succeeded. Both very high water vapor losses and very low water vapor losses through crusts have been measured. From the slope of the upper curve of Figure V.5, "T", - such a high IWVL is always found following spontaneous removal of the crust - it may be concluded that the skin is still in the phase of the formation of a temporary barrier (10 to 15 days after the severe injury). The horny cell layer is parakeratotic and is shed by chapping some days later. Thus it is seen, that the entire regeneration process, following the removal of the temporary barrier T, appears to be very much the same as the one dealt with just before (removal of only the horny layer), except that the IWVL persists for a much longer period (upper curve of Figure V.5).

It is suggested that measurements of the IWVL may be used after injury to the skin in order to determine the extent of the original injury. Reference to Figure V.5 shows that it is not even necessary to make these measurements immediately

after the injury, since the regeneration follows a reproducible pattern in each case. The model of Figure V.5 is based on several determinations of the IWVL at many individuals (SPRUIT, 1965; SPRUIT & MALTEN, 1965; 1966; 1968; MALTEN & SPRUIT, 1966; MALTEN et al., 1968; BAKER & KLIGMAN, 1967; MATOLTSY et al., 1962).

CHAPTER VI

INJURY BY ALKALI

Summary

The measurement of the rate of alkali neutralization (ANrate) of the skin has recently been improved and can now be added to the so-called alkali neutralization test (AN-test) and alkali resistance test (ARtest) which were introduced by BURCKHARDT (1935, 1947 respectively).

In the present work a number of experiments were carried out with this new technique, in particular to investigate the effect of the pH upon the ANrate. The results of these experiments showed clearly that two independent processes were occurring.

a: At pH values between 7 and 10, the ANrate was comparatively low; it was about linearly related to pH, and the skin suffered no permanent damage. These observations suggest that the process occurring here is simply the neutralization of CO_2 transported through the skin.

b: At pH values above 11, the ANrate was greatly increased; damage to the stratum corneum was demonstrated. This process is believed to be a direct reaction between OH^- ions and the material of the stratum corneum.

Additional experiments were carried out to investigate the regeneration of the water barrier after exposure to high pH's. A good correlation was obtained with the results found after stripping; exposure to pH 11.7 for 1 hour produced damage identical to that resulting from total removal of

The contents of this chapter will be published in "Current Problems in Dermatology", Vol. 3. Courtesy of S. KARGER AG, Basel.

the stratum corneum. This critical pH was increased by application of vaseline.

The skin adapted to a daily exposure to an injuring concentration of the alkaline solution; an example of such an experiment is given.

VI.1 ALKALI NEUTRALIZATION TEST AND ALKALI RESISTANCE TEST

In the literature and in the routine testing of the ability of the skin to resist irritant materials, the so-called alkali neutralization test (ANtest) (BURCKHARDT, 1935) and the so-called alkali resistance test (ARtest) (BURCKHARDT, 1947) gained appreciable interest for several years. BURCKHARDT standardized the alkali exposures empirically, and used as his criteria either the colour change of an indicator or the development of a clinically discernable skin lesion. He attempted to correlate the results of both tests with groups of patients suffering from various skin diseases in order to check whether these tests could be of any help in elucidating the pathogenic factors in the various groups. The most important test conditions are presented in Table VI.A.

In the alkali neutralization test (ANtest) as proposed by BURCKHARDT in 1935, a small amount of 0.006 N NaOH contacts the skin until, after a few minutes, a colour change of the phenolphthalein indicates the end of the first exposure. The procedure is repeated on the same area of skin 10 times in all. At the beginning of the exposure to NaOH the pH of the solution is about 12; at the end it is 8.3. The object of the experiment is to determine whether the skin maintains its capacity to neutralize this amount of alkali within a certain time limit. Clinically this kind of exposure does not injure the skin. By measurement of the water vapor loss, however, SPRUIT & MALTEN (1968) demonstrated

Table VI.A:

Characteristics of alkali neutralization test (ANtest) and alkali resistance test (ARtest).

	alkali neutralization test (ANtest)	alkali resistance test (ARtest)
alkaline solution applied to the skin: (1 drop = 1/30 ml)	1 drop 1/80 N NaOH + 1 drop phenolphthalein	1 drop 1/2 N NaOH
pH at the beginning:	12	13
test termination:	at pH 8.3	1: after 10 minutes 2: after 2 x 10 min 3: after 3 x 10 min
criteria:	colour change	skin reaction
exposed skin area:	6 cm^2	3 x 6 cm^2
procedure to be repeated:	10 x	1 x
test result good:	all within 5 minutes	no skin reaction
,, ,, normal:	within 5 - 7 minutes	no. 3: + reaction no. 2: no reaction
,, ,, bad:	once exceeding 7 minutes	no. 3: ++ reaction no. 2: + reaction
literature reference:	BURCKHARDT(1935)	BURCKHARDT(1947)

Combination of neutralization and resistance tests:
ANtest applying three different NaOH concentrations at three sites of the skin (VERMEER et al., 1954).

that this exposure does, in fact, slightly damage the water barrier of normal, healthy skin.

In the alkali resistance test (ARtest) the initial concentration of the alkaline solution is 80 times stronger than in the ANtest. At the beginning of the exposure, the pH of the alkali is about 13: this is really harmful to skin. Because of the neutralizing capacity of the skin, the pH soon falls to lower values. The test is simultaneously carried out at three sites of the skin. At the first site, the test is not repeated and the alkali exposed once only for ten minutes. No clinical reaction (papules, vesicles) results from this ten minutes exposure, even with a skin of low alkali resistance. At a second test site, the resulting liquid is blotted from the skin and another drop of 0.5 N NaOH applied to the same site for another ten minutes. Normally, no skin reaction (papules, vesicles) is observed. However, a delicate skin may react slightly. At a third site, the resulting liquid is again blotted from the skin and a third drop of 0.5 N NaOH applied to the same site for yet another ten minutes. An easily injured skin now reacts very clearly and even a normal skin may react slightly. The reactions are judged by eye immediately after the test, and again 24 hours later.

In order to reduce the difficulty of interpreting and correlating the results of the ANtest and the ARtest, a combination of both tests was developed by VERMEER et al. (1954). In the meantime several attempts were made to develop a potentiometric titration (KOCH, 1939; PIPER, 1943; MENEGHINI, 1960; LOTMAR, 1964; SPIER & BEIERSDORFF, 1964). However, it is only recently that these attempts have proved more successful; a useful titration method was published by SCHUTTER (1965), soon followed by TRONNIER (1966) and by SPRUIT & MALTEN (1968), all investigators employing the same automatic titrator of RADIOMETER ex Copenhagen. These investigations introduced a new measurable characteristic of the skin; the alkali neu-

tralization rate (ANrate).

VI.2.1 ALKALI NEUTRALIZATION RATE

The alkali neutralization rate (ANrate) is the rate at which the skin is able to change the pH value of an aqueous solution placed upon the skin surface. As this rate is dependent on the pH of the solution, the measurement can only be carried out by titrating additional alkali to the solution in order to maintain a constant pH (so-called titration at pH-stat). The scheme of the instrumentation is represented in Figure VI.1.

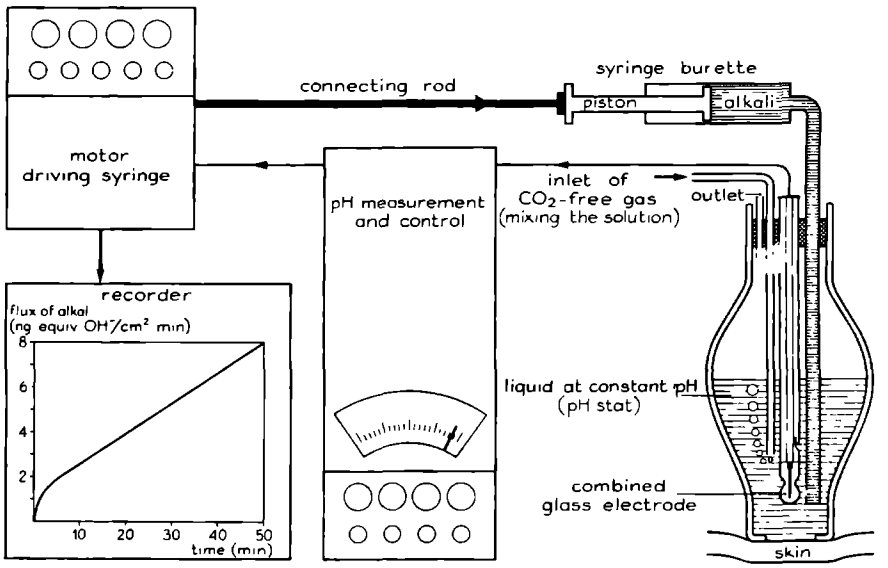


Figure VI.1:
Scheme for the measurement of the alkali neutralization rate (ANrate) at pH-stat.

The pH of the liquid in contact with the skin is automatically kept at a predetermined constant value. The pH is measured by means of a glass electrode. If this pH decreases, a con-

centrated alkaline solution is automatically driven from a burette into the solution by a motor. The amount of titrated alkaline solution is recorded. The rate of addition of alkali which is needed to maintain a constant pH, is used as the criterion for the neutralizing capacity of the skin. In the initial period of about 5 minutes the titration proceeds rapidly; during these first few minutes apparently some excess acid, present on the skin surface, is neutralized. After this initial period a constant ANrate is obtained. A steady state can be recorded during an hour or more (at pH below 11.8).

The titrated alkali per unit of time (during the steady state) has been called:
a: "alkali neutralization capacity" by SCHUTTER (1965),
b: "consumption of N/1000 NaOH cm^{-2} h^{-1}" by TRONNIER (1966),
c: "alkali neutralization rate" by SPRUIT & MALTEN (1968).
It is determined in equivalents of alkali per cm^2 skin and per min. Physically a flux of alkali is supplied to the solution above the skin in order to maintain the original pH.

In the ANtest, the ARtest, and the measurement of the ANrate, free exchange of gases with the environmental air is hampered because the skin is covered by an alkaline solution. Carbon dioxide is bound by the alkaline solution; this factor will be considered closely in some following paragraphs.

VI.2.2 ALKALI NEUTRALIZATION RATE DEPENDENCE ON pH

The ANrate changes with variation of the pH-stat (Figure VI.2). The measurements of TRONNIER (1966), SCHUTTER (1967), and SPRUIT & MALTEN (1968), are not identical, though the differences are minor. The experimental circumstances of these investigations were different; SCHUTTER cleaned the skin of healthy volunteers in a standardized way with alcohol and with

HCl before each measurement, and reports that the alkali flux increased slowly and nearly linearly at pH values from 8.5 to 10, but more rapidly at higher pH (10.5). The alkali flux measured by SPRUIT & MALTEN was only a little greater andshowed the same trend, though they stated that a greater rate of change of the flux was observed at pH values above 11.2 in un-

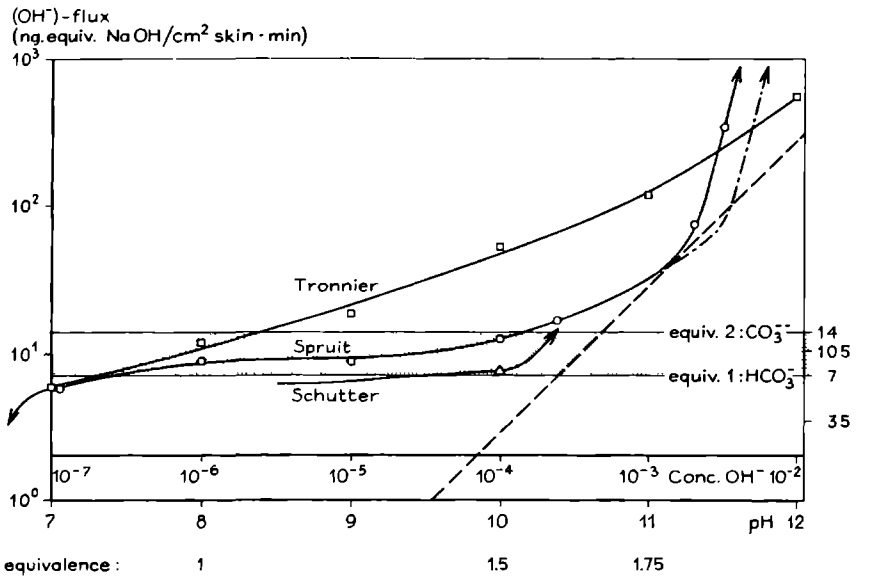


Figure VI.2:
The alkali neutralization rate (ANrate) of human skin in vivo, estimated as a flux of alkali (OH^- flux) at various values of the pH-stat. Measurements of TRONNIER (1966), of SPRUIT & MALTEN (1968), and of SCHUTTER (1965, 1967).

cleaned normal skin and somewhat higher in vaseline pretreated skin (Figure VI.2, curve .-.-.-.). TRONNIER reported measurements on the skin of pateints and healthy volunteers. The flux measured by TRONNIER was identical to the other investigators at pH 7: however, it was somewhat greater at pH values of 8 - 11. and he did not report a clear discontinuity of the curve at high pH values. Both TRONNIER (1966) and SPRUIT & MALTEN

(1968) emphasize that the alkali neutralization proceeds with a constant flux with respect to time, not only at low pH, but also at a pH 12.

At pH > 10.6 hydrogen bonds are broken in some smaller proteins; denaturing of the more highly polymerized proteins is supposed to occur above pH 11.5 (ERLENMEYER et al., 1968; ZIMMER, 1968). A pH of 11.5 has been shown to be the minimum value which will damage the skin (MATOLTSY et al., 1968). Moreover, it has been demonstrated by SPRUIT & MALTEN (1968) that the critical pH value of 11.2 at the discontinuity in their rate curves coincides with that required to injure the skin. It can therefore be concluded that another mechanism of the alkali neutralization is involved at harmful pH values (>11.5), compared with non-harmful pH values (< 10). The precise pH value at which this transition occurs depends on a number of factors, including individual variation, seasonal factors, pretreatment, condition of the skin, etc..

An acid pH is not neutralized nor even changed by the skin as estimations of SCHUTTER (1967) have proved.

VI.2.3 ALKALI NEUTRALIZATION RATE MECHANISM AT LOW pH (7 - 10)

The rate of neutralization at pH 3.5 is too low to be determined (SCHUTTER, 1967), despite the fact that the H^+-ion concentration difference between the outside and the inside of the skin is greater at pH 3.5 than at higher pH values. Therefore, the H^+-ion does not permeate through the skin significantly (as compared with the ANrate), and cannot contribute to the alkali neutralization by subsequently reacting with OH^--ions, unless the skin membrane is altered in its characteristics.

Neither does the OH^--ion diffuse through the skin boundary into the inside milieu. If it is postulated that at pH 11 all

alkali were neutralized by diffusion of OH^--ions into the interior skin, the ANrate being 30 ng equiv NaOH cm^{-2} min^{-1} (1 ng equiv = 10^{-9} g equiv), then the ANrate would have been 3 ng equiv NaOH cm^{-2} min^{-1} at pH 10 and only 0.3 ng equiv NaOH cm^{-2} min^{-1} at pH 9 (see Figure VI.2, curve - - -), because the rate is linearly dependent on the concentration difference according to FICK's law of diffusion. Therefore, the transport of OH^--ions through the skin cannot contribute significantly to the ANrate.

Many acid substances are present at the surface of the skin at the moment when the alkali is applied to the skin. These acid substances are neutralized by the alkali and cause an increased ANrate at the beginning of the experiment, as has been recorded in the graph of Figure VI.1. This increased ANrate persists for only five minutes. Acid valencies originating from the sweat (ROBERT & JADDOU, 1942; WOHNLICH, 1948), lactic acid (ZOON et al., 1956), amino acids (VERMEER et al., 1954), water-soluble substances of the stratum corneum (SPIER & PASCHER, 1956), and the protein of the horny layer itself (BURCKHARDT, 1935; JACOBI, 1942; PIPER, 1943; SCHULZE, 1943; SPIER & BEIERSDORFF, 1964) can all contribute to the increased ANrate at the beginning of the measurement. They do not influence the ANrate after this initial period (TRONNIER 1966). Moreover, it has been shown that using cadaverous human skin of the back, the ANrate is less than 0.5 ng equiv NaOH cm^{-2} min^{-1}, thus providing confirmation of this thesis.

It has been postulated that CO_2 contributes appreciably to the alkali neutralization (PIPER, 1943; SCHULZE, 1943; LOTMAR, 1964). It has been proved recently by SCHUTTER (1967) that at moderate pH values (8 - 10.5) alkali neutralization is mainly the result of transport of carbon dioxide through the skin. The contribution of CO_2 to the neutralization process was estimated to be between 60 % and 98 %; this became considerably

smaller at pH values above 10. The reactions which can take place when carbon dioxide passes from the skin and to the titration vessel, are presented schematically in Figure VI.3.

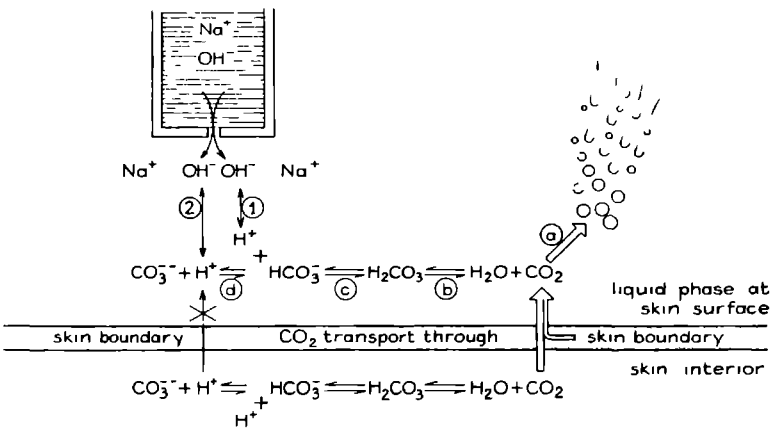


Figure VI.3:

Aspects of the transport of carbon dioxide through the skin are:

a: skin's buffering capacity by constant CO_2 flow is possible because of its volatilization, step "a";

b: skin's neutralizing capacity of alkali may be realized as a result of the hydration of CO_2 to carbonic acid, step "b".

At pH 4 the reaction proceeds only to H_2CO_3: excess CO_2 simply volatilizes along "a". At pH 8 the reaction proceeds as far as step "1": the equivalence of the neutralizing reaction of CO_2 being one. At pH 10 the reaction proceeds along both step "1" and step "2", as described in the text.

The amount of alkali that has to be supplied to the titration vessel in order to maintain a constant pH during the transport of CO_2 through the skin is dependent on the pH. This can be understood from the following considerations. The constant for the neutralization of carbonic acid, pK_1, is about 6 and the constant pK_2 is about 10 (see Figures VI.3 and 4). Thus equal amounts of H_2CO_3 and HCO_3^- are present at pH 6, so that each two moles CO_2 require one equivalent of alkali in order to maintain the pH at 6, and hence the alkali equivalence of the neutra-

lization of 1 mole of CO_2 is 0.5 at pH 6. At pH 10, equal amounts of HCO_3^- and CO_3^{--} are present, and 2 moles of CO_2 now require 3 equivalents of alkali in order to maintain the pH at 10. The neutralization equivalence of 1 mole CO_2 is therefore 1.5 at pH 10. In Figure VI.4 the alkali equivalence of CO_2 at constant pH is presented as a function of the pH-stat.

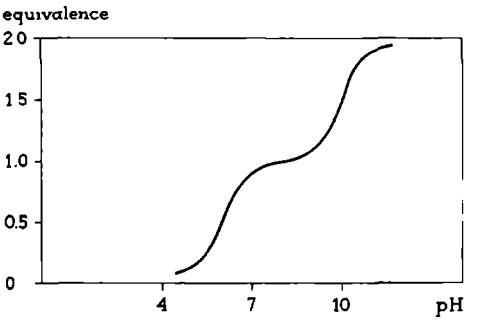


Figure VI.1:
Alkali equivalence of the neutralization of one molecule of carbon dioxide as a function of the pH-stat.

It has been estimated that, on the average, the CO_2 loss from human skin is about 100 ml CO_2 gas per m^2 skin and per h, equivalent to 7 ng mole CO_2 cm^{-2} min^{-1} (SHAW & MESSER, 1930; SCHULZE, 1943; ROTHMAN, 1954). The same amount of CO_2 flux must occur when the ANrate is determined at any particular pH. At pH 8 the equivalence of the CO_2 neutralization reaction is about one, so that 7 ng mole CO_2 corresponds to 7 ng equiv CO_2 or OH^-. This level is shown in Figure VI.2 (equiv 1: HCO_3^-) and corresponds roughly to the results of the experiments of SCHUTTER, TRONNIER, and SPRUIT & MALTEN, mentioned before. At pH 10 the equivalence of the CO_2 neutralization is about 1.5, so that the same 7 ng mole CO_2 corresponds to 10.5 ng equiv CO_2 or OH^-. This level is also indicated in Figure VI.2 as is the maximum level at an equivalence 2, corresponding with 14 ng equiv CO_2 or OH^- (equiv 2: CO_3^{--}). The measurements of

SCHUTTER and of SPRUIT & MALTEN fall within this darkened area between 7 and 14 ng equiv OH^- of Figure VI.2 between pH 7 and pH 10. From this it can be concluded that the ANrate at pH values up to 10 is mainly (or even exclusively) governed by the CO_2 flux from the skin, in agreement with the experimental observations of SCHUTTER (1967). The measurements of TRONNIER (1966) do not deviate appreciably up to pH 9; however, they tend to be increased above the measurements of the other investigators at higher pH (9 - 10).

VI.2.4 ALKALI NEUTRALIZATION RATE: MECHANISM AT pH (10 - 11.5)

Theoretically the CO_2 flux from the skin should cause the ANrate at pH 11.5 to be only a little increased above the neutralization rate at pH 10, as the equivalences of the reaction are respectively about 1.9 and 1.5. However, a much greater increase in the ANrate is, in fact, found at higher pH values in this range. The CO_2 flux from the skin cannot therefore be the only factor governing the ANrate.

Contrary to the agreement in results of SCHUTTER (1965), TRONNIER (1966) and SPRUIT & MALTEN (1968) at pH 7 - 10, these investigators differ significantly in their results at pH 10 - 11.5, as is evident from Figure VI.2. SCHUTTER did not investigate at pH above 10.5 because it would injure the skin. SPRUIT & MALTEN indicated that the skin was not visibly injured at pH values up to 11.2 and showed that the effects of the damage started above this pH value. Both SCHUTTER and SPRUIT & MALTEN showed that the pretreatment and the condition of the skin are important factors in the measurement of the ANrate at higher pH values. SCHUTTER (1967) demonstrated that not only carbonates are found in the solution covering the skin; soluble reaction products result from the contact of the skin with the

more alkaline solution. The mechanism of the ANrate is no longer a simple neutralization but starts to include chemical changes of the stratum corneum. The different results of the various authors in this higher pH range may thus originate from different ways of pretreatment of the investigated skin and its condition prior to the measurement.

VI.2.5 ALKALI NEUTRALIZATION RATE MECHANISM AT HIGH pH > 11.5

At pH 11.5 and higher, the ANrate rises still more rapidly, and the skin is usually visibly damaged by the alkaline solution after an hour's exposure. The ANrate is still a constant function of time, however, up to pH 12. Above pH 12 the measurement becomes increasingly difficult, as blisters appear within a period of an hour.

The results indicate that the alkali is reacting with the horny layer material. The chemical change results in a skin barrier of higher permeability as is evident from the increased water vapor loss. It seems possible that in this pH range the ANrate is determined mainly by the rate of this reaction between OH^--ions and the horny layer material. Obviously some CO_2 transport still occurs, but it is hardly likely that it increases sufficiently to make an appreciable contribution to the observed values of ANrates at pH values over 11.5.

The horny layer material can be screened from the injuring influence of the alkali to some extent, e.g. by a thin film of vaseline, which causes a reduction of the ANrate (see Figure VI.2, curve .-.-.-.).

VI.3.1 ASSESSMENT OF THE INJURY BY ALKALI BY MEANS OF THE

ALKALI RESISTANCE TEST

In the ARtest the skin is injured by a standardized technique, as indicated in Table VI.A, and described in Section VI.1. The amount of alkali applied to the skin in the ARtest has been increased to such an extent that a reasonably short exposure to alkali is capable of producing an injury. The injury becomes clinically visible in a short time and is judged qualitatively. The histological reaction has recently been described by BANDMANN (1968). The influence of the pretreatment of the skin is not very important because the alkaline solution is applied three times.

VI.3.2 ASSESSMENT OF THE INJURY BY ALKALI BY MEANS OF THE INCREASE OF THE WATER VAPOR LOSS

When alkali is applied to the skin, its water vapor loss is increased as soon as the water barrier (located in the horny layer) is injured. The increase of the water vapor loss (IWVL) can therefore be an indication of the degree of the injury. Supplementary information can be obtained from water vapor loss measurements during the regeneration of the injured skin, as became evident from the results of Chapter V. The IWVL was measured daily for at least a month. Some of these measurements are illustrated graphically in Figure VI.5. For the sake of clarity the individual points have been omitted from this graph.

The experiments of Figure VI.5 are the same ones as those of Figure VI.2, curve "SPRUIT". The skin has been exposed to alkali for 1 hour and the IWVL estimated one day later and at daily intervals afterwards. An exposure to alkali at pH

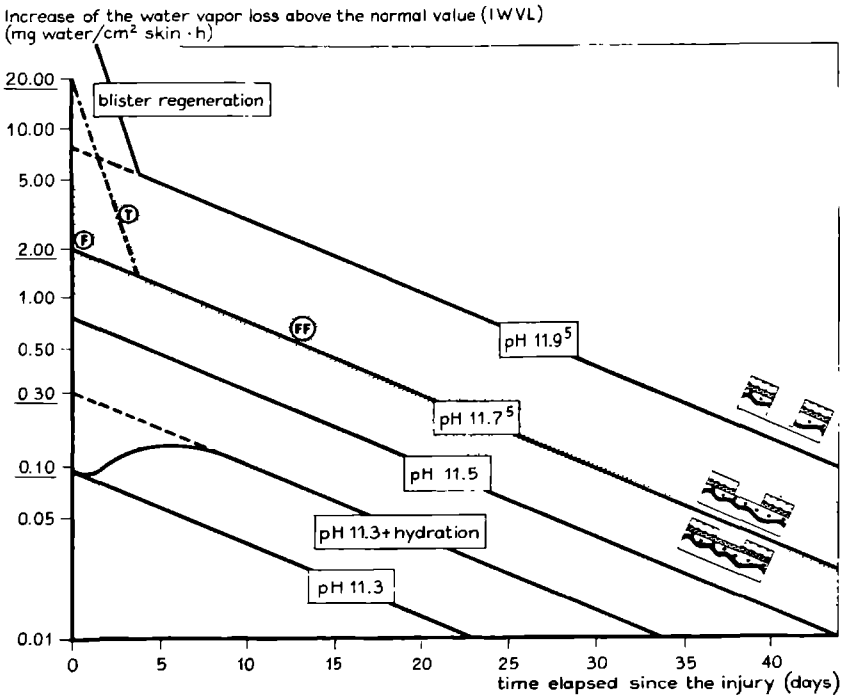


Figure VI.5:

Regeneration of forearm skin following an exposure to a solution at the indicated pH for one hour, as estimated from the increase of the water vapor loss (IWVL) of the skin. The extent of the injury can be estimated by extrapolating to time 0 from a curve recording the formation of a "final" barrier ("FF" and "F"). "Temporary" barrier formation is indicated by "T". The gray area indicates the result of an injury equivalent to the complete removal of the stratum corneum, and was achieved by exposure to pH-stat 11.75 for one hour. The injury resulting from other pH-stat exposures is indicated by relevant recordings of the IWVL. (Figure derived from a publication in Berufsdermatosen 16: 11-24, 1968)

11.3 was shown to cause an initial IWVL of 0.10 mg cm^{-2} h^{-1}; an identical effect to that obtained by stripping half the horny layer with adhesive tape. When this same skin was subsequently exposed to water for 2 hours on the day after the alkaline injury was carried out, the measurements resulted in the curve "pH 11.3 + hydration". This additional, normally non-injuring exposure caused a severe injury to the skin, corresponding to an extrapolated IWVL (at time 0) of 0.30 mg cm^{-2} h^{-1}. The regeneration of the skin is clearly retarded for ten days.

The skin was also exposed to alkali for 1 hour at pH 11.75, and the IWVL measured the next day and on several following days. It is seen from curve "FF" that exposure to alkali at this pH caused an initial IWVL of 2.00 mg cm^{-2} h^{-1}. This degree of injury and the subsequent regeneration rate are both identical to those obtained after complete removal of the horny layer (and maybe a part of the stratum granulosum) by stripping the skin with adhesive tape: this has been indicated by the gray area of Figure VI.5.

Measurements of the IWVL during the first four days are often much increased above the values of curves such as "F", and instead follow curve "T". The same phenomenon has been described following the stripping of the horny layer. The very high IWVL again induces the formation of the so-called "temporary" barrier, parakeratotic in nature (see Chapter V), which is shed in scales about five days afterwards. After the shedding of this temporary barrier, "final" layer formation results as before.

At pH 11.95 blisters result when the alkali is exposed for 1 hour. The blistered skin regenerates with the same speed as a temporary barrier formation (identical slope of the curve). Again, the degree of the injury is related to the final barrier formation. An IWVL, extrapolated to time 0, increased above 5 mg cm^{-2} h^{-1}, indicates that the injury extended at least to the

stratum spinosum.

VI.4 RESPONSE OF THE SKIN TO REPEATED INJURY BY ALKALI

The skin can get used to exposures of alkali. In order to investigate such an adaptation the skin has to be exposed several times to the alkaline agent. An identical exposure to the same amount and concentration of alkali may be repeated each day for about a week or more. Such investigations have been carried out by SPRUIT & MALTEN (1968); Figure VI.6 is derived from this report. In this experiment the same volunteer was exposed to 0.03 N NaOH (not at pH-stat) for one hour each day immediately following a measurement of the IWVL at the exposed site of the skin. All exposures to this alkali treatment resulted in the same ultimate injury, whether the investigations were carried out in summertime (August) or in autumn (October), as is seen from the final barrier regeneration curve "F". Whether the skin was exposed six times or nine times made no difference either; the additional three exposures did not aggravate the injury. Yet there is a marked difference between the summer-skin and the autumn-skin of this volunteer as far as the reaction to the first exposures to the alkali is concerned. Under the environmental conditions of August 1966 a "temporary" barrier ("T") was formed following a high initial IWVL. Such temporary barrier formation is not evident from the curves of October 1966. Surprisingly, however, scaling of the skin following the exposures did, in fact, occur in October. A parakeratotic skin barrier was formed, and this barrier layer impeded additional injury by repeated exposures to the alkali. This impedance to additional injury can be considered as a response of the skin to changed environmental circumstances. The injury caused the formation of a mechanically inferior, easily scaling skin, which, however,

physiologically improved the protection against a repeated insult by the alkali.

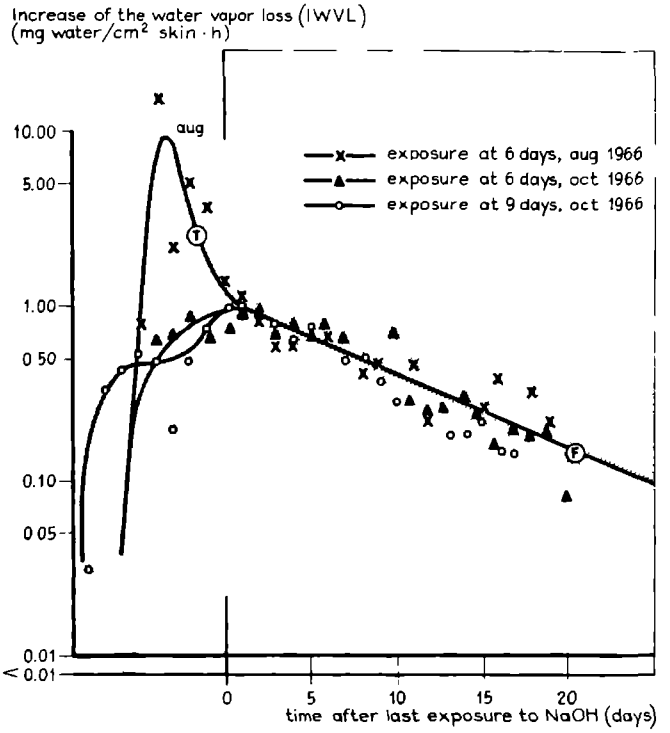


Figure VI.6:

Injury to and regeneration of the water barrier of forearm skin after exposure to 0.5 ml 0.03 N NaOH per cm^2 skin for one hour on successive days. Final barrier formation ("F") was identical in different ambient circumstances; temporary barrier formation ("T") shows clear adaptation of the skin to subsequent alkali exposures in August.

(Figure derived from a publication in Berufsdermatosen 16: 11-24, 1968)

CHAPTER VII

INJURY BY ORGANIC MATERIALS

Summary

The horny layer must protect the skin against the entrance of harmful substances. In this Chapter it has been shown that measurements of the water vapor loss during and after repeated exposures to such substances may be used to obtain information about the protective qualities of the horny layer during such an attack and during the following regenereation period.

Human skin was exposed in vivo for one hour on 6 successive days to ethanol, ethylacetate, methylethylketone. toluene and for 15 minutes on 6 successive days to acetone, chloroform, trichlorethylene, dimethylsulfoxide and rectified light benzine. These exposures resulted in damage to only the horny layer; the effect of the last two solvents was more severe than that of the others. Following a more severe injury the skin responds with the formation of a "temporary barrier" exactly as was described in previous sections.

Six daily one hour exposures to 13.5 % and to 18 % phenol concentrations in resorcinol-formaldehyde resin resulted in the same injury as exposures to 2 % aqueous phenol or 0.03 N NaOH. Exposure to 18 % phenol in resin resulted in the formation of a more effective temporary barrier than 13.5 % phenol in resin. The protective adaptation, however, failed when the phenol concentration appreciably surpassed 18 %. The practical implication of these findings, particularly

The contents of this chapter have been published in Berufsdermatosen 16: 135-147 (1968). Courtesy of the editor of this journal.

as applied to occupational hazards in industry. have been discussed.

VII.1 INJURY BY ORGANIC SOLVENTS

Solvents, whether polar or nonpolar, or both polar and non-polar, extract soluble substances from the skin (SCHOENHERR, 1961; ROMITI & SZAKALL, 1964; BULLOUGH & LAURENCE, 1964). As a consequence especially the colloidal structure of the skin is changed and the water content of the horny layer influenced by ambient atmospheric circumstances, resulting in a more easily chapped horny layer. Until recently, only clinical observations could be used to study the effects of the various industrial solvents.

By measuring quantitatively the damage to the water barrier function and its subsequent regeneration, a better insight into the consequences of exposure to various solvents can be obtained. In order to imitate as far as possible the typical, real-life circumstances, the skin of the volar aspect of the forearm of a volunteer was exposed to the solvent on each of 6 successive days, in the same way in which the skin was exposed to an alkaline solution (Section VI.4). The non-polar solvents were applied to the skin in glass cylinders with an opening of 2 cm^2, fixed to the skin by agar gel; the polar solvents were applied to the skin in plastic cylinders, fixed by nobecutane, like the NaOH (see Chapter V). The contact time for ethanol, ethyl-acetate, methyl-ethyl-ketone (MEK) and toluene was 60 minutes per day; for acetone, chloroform, tri-chlor-ethylene (TRI), rectified light benzine and di-methyl-sulfoxide (DMSO) it was 15 minutes per day.

A number of sites to be exposed to solvents were marked on the volar aspect of the forearm by circles with a ballpoint pen and a few parallel sites marked as controls. The determination of the normal water vapor loss of all these sites preceded

the exposure to solvents. The next day, these measurements were repeated in order to determine the increase of the water vapor loss (IWVL) resulting from the exposure. Thereafter, subsequent exposures were carried out until a total of six exposures was completed.

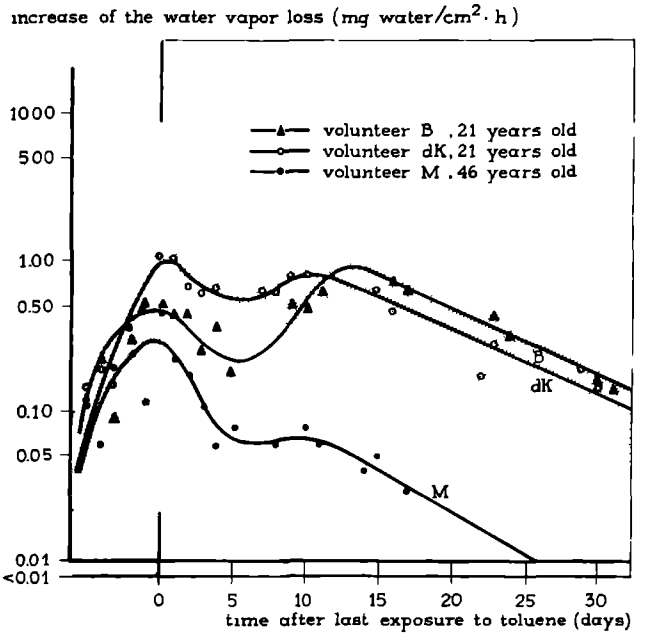


Figure VII.1:
Injury to and regeneration of forearm skin after exposure to toluene for one hour on six successive days.

The investigation of the injury and the regeneration of the skin following exposures to toluene (Figure VII.1) and to ethanol (Figure VII.2) have been carried out and represented in the same way as before (Chapters V and VI). It can be concluded from these graphs that the horny layer of the skin of the two younger volunteers B and dK had to be renewed completely. Some difference was observed between the effects of the non-polar toluene and the more polar ethanol with respect to the temporary barrier

formation; this was more evident after exposure to toluene. The older volunteer, M, reacted differently to the younger ones, especially in that the toluene was less destructive to his skin. Toluene did not injure the horny layer of this subject at all; ethanol, however, resulted in slight damage.

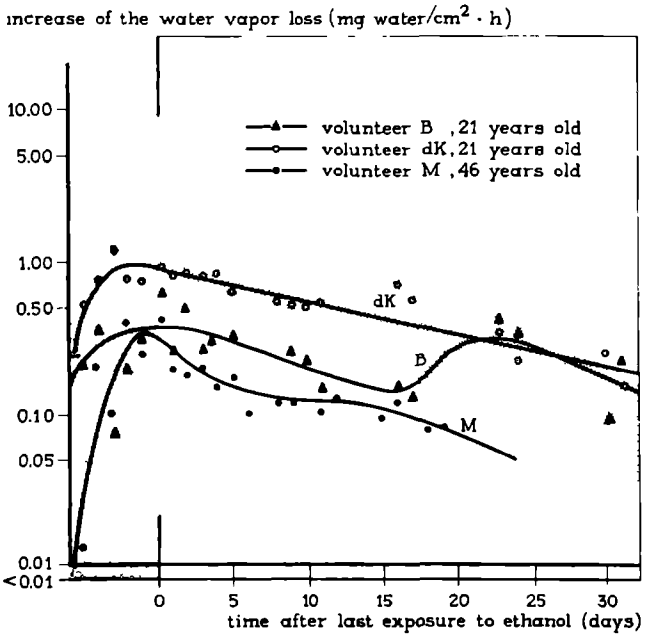


Figure VII.2:
Injury to and regeneration of forearm skin after exposure to ethanol for one hour on six successive days.

Exposure to ethylacetate and methylethylketone resulted in injuries similar to those caused by ethanol, and with the same kind of regeneration.

It has been demonstrated that dimethylsulfoxide (DMSO) increases the penetration of several substances through the skin (KLIGMAN, 1965; SWEENEY et al., 1966). DMSO has even been used therapeutically because of this effect. For safety's sake, the duration of the experimental exposure was therefore

limited to 15 minutes instead of the usual 60 minutes. In spite of this limitation, the injury appeared to be quite severe, especially on the skin of the younger volunteers (Figure VII.3). DMSO will presumably increase the penetration of other substances because of the damage which is caused to the stratum corneum. If this is

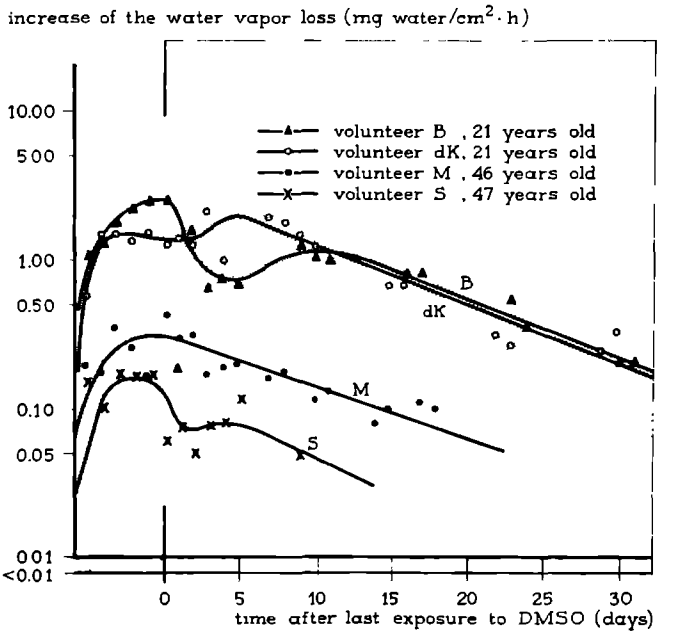


Figure VII.3:
Injury to and regeneration of forearm skin after exposure to dimethylsulfoxide (DMSO) for 15 minutes on 6 successive days.

so, the extent of this increase will not be predictable, as the two older volunteers M and S showed much less injury to their skin following the exposure to DMSO than the two younger volunteers B and dK.

The exposure to rectified light benzine (often used for cleaning the hands in the engineering branch) resulted in macroscopically visible injury to the blood-vessels (petechiae), although the exposures were limited to 15 minutes per day. The exposures

were followed by the formation of a temporary barrier, decreasing the water vapor loss very effectively (Figure VII.4). This temporary barrier lasted quite a long time, and the water vapor loss was not only very effectively limited during the first week after the exposures, but sometimes it was even less than the controls (see Figure VII.4; values at 4, 5 and 6 days after the last exposure). However, as soon as the temporary barrier was shed (about 10 days after the last exposure to the solvent), the final barrier showed a normal regeneration pattern. Not all volunteers, however, behaved like the one of Figure VII.4.

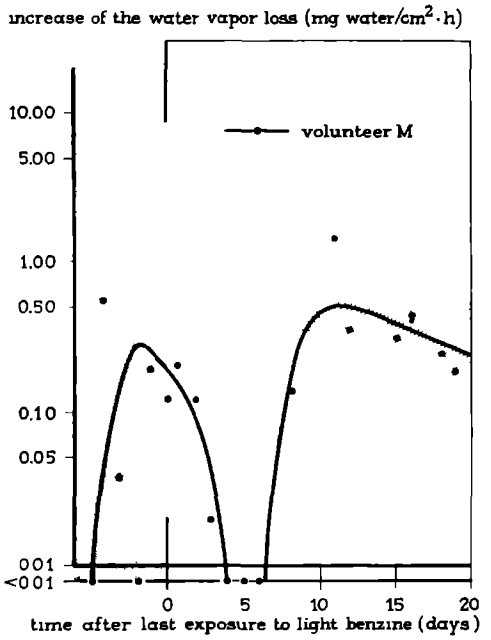


Figure VII.4:
Injury to and regeneration of forearm skin after exposure to rectified light benzine for 15 minutes on 6 successive days.

Exposures for 15 minutes to chloroform and trichlorethylene resulted in injuries equal to, or somewhat higher than, those of 1 hour's exposure to ethanol, and were followed by the

same kind of regeneration. The exposures for 15 minutes to acetone produced very little effect; the IWVL did not surpass 0.5 mg water cm^{-2} h^{-1}.

VII.2 COMPLICATED INJURY BY AN INDUSTRIAL HARDENING CHEMICAL

Following 6 exposures of 1 hour each day for 6 successive days (the standardized injury), similar results were obtained following the application of:

a: 0.03 N NaOH,

b: 2 % phenol in water solution, and

c: 15 % phenol in resorcinol-formaldehyde resin.

From this simple statement it is obvious that a protecting influence is afforded by the resorcinol-formaldehyde resin. Whether this is related to the distribution coefficient of phenol between the skin and the resin (see BLANK, 1964) or due to the formation of a protective resin layer over the horny layer cannot yet be concluded.

The injury to the skin and its regeneration following exposure to two different phenol concentrations in the resorcinol-formaldehyde resin, 13.5 % and 18 %, is represented in Figure VII.5 in the usual way. These experiments were carried out upon the forearm of two volunteers of the same age, 21 years old. Both experiments ran a similar course.

The first 2 or 3 exposures did not result in an increase of the water vapor loss; on the contrary, the water vapor loss even slightly decreased. The well-known effect of sweat-pore closure by phenol and the influence of the resin on the stratum corneum may both have contributed to this decrease (DOBSON & LOBITZ, 1957). Though these first exposures seemed harmless.

they certainly were not. This was confirmed in another experiment, in which only two exposures sufficed to obtain an injury of the horny layer; the damage, however, became obvious only after a latent period of about 2 weeks. During the last few of the six exposures to phenol in resin, the water vapor loss increased most at the site exposed to the higher (18 %) phenol concentration.

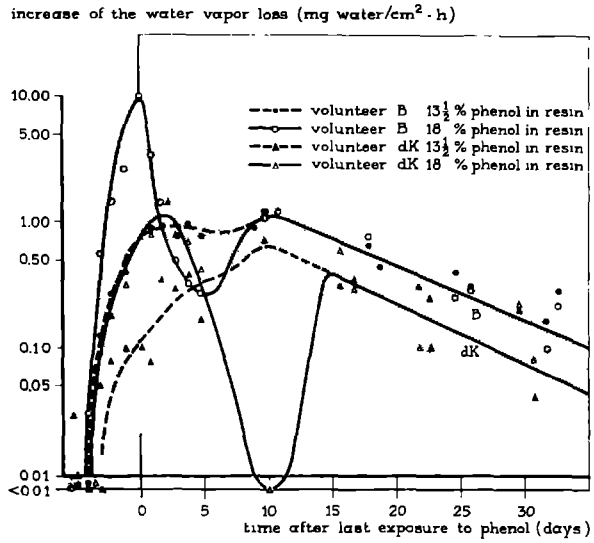


Figure VII.5:
Injury to and regeneration of forearm skin after exposure to industrial phenol in resorcinol-formaldehyde resin for one hour on 6 successive days.

From the curves obtained at the concentration of 18 % phenol in resin, it is obvious that a temporary barrier laver was formed; the regeneration proceeded very rapidly, immediately following the exposures. The temporary barrier was shed after one or two weeks. The temporary barrier formation using a concentration of 13.5 % phenol in resin was not so evident as in the case of the higher phenol concentration, although even at the lower

concentration the curves indicate some degree of barrier formation.

The final barrier formation occurring after exposure to both concentrations, 13.5 % and 18 % phenol in resin, was identical after the temporary barriers had been shed. It appears obvious that the formation of a more effective temporary barrier layer at the higher phenol concentration had prevented a more severe final injury of the skin. The temporary barrier formation, therefore, can be considered as an effective response of the skin to the attack by a chemical, when the concentration of the chemical is sufficiently high.

In these experiments the skin was rinsed with tapwater immediately following the exposure to the phenol in resin solution, thus obviating stripping of the skin by the hardened resin. In occupational circumstances, situations are common in which the resin remains upon the skin for many hours and "cures" on the skin before it is removed; in this way the skin will be stripped. Complaints from workmen afterwards are very understandable. It therefore surprises that, under certain circumstances, a 19 % phenol concentration in resorcinol-formaldehyde has been used occupationally without causing any complaints. In this case the response of the skin by the formation of a temporary barrier is presumably a sufficient protection. This response of the skin is its natural defence and may be accepted as such.

A 22 % phenol in resin concentration was found to be necessary by another industry for a new application of the material. A short time after the production had started, complaints were received. It is therefore evident. that in occupational as well as in experimental circumstances a critical concentration really exists, which should not be surpassed, without protective measures.

In an experimental exposure to 22 % phenol in resin for one hour on each of two successive days a slight increase

of the water vapor loss was observed on the day following the first exposure. and a decrease of the water vapor loss was observed on the day following the second (and final) exposure. At this time and the next day, no injury was evident from the measurements. However. by the following day the injuring effect of this 22 % phenol concentration manifested itself very clearly, with an IWVL of 1.0 mg cm^{-2} h^{-1}; this afterwards regenerated following the final barrier pattern. Thus, compared with exposures to 13.5 % and 18 % phenol in resin (Figure VII.5), this injury manifested itself earlier, and it would undoubtedly have been been more severe, if the daily exposures had been continued for more than two days. The critical value of the phenol in resin concentration has thus been surpassed also under the experimental conditions.

A pretreatment of the skin with vaseline proved to have a protecting effect during the two exposures to 22 % phenol in resin and for at least two weeks afterwards. Obviously the protection of the skin can be improved.

SAMENVATTING

I Sinds vele jaren heeft men diverse faktoren nagegaan, die van invloed zijn op het verlies van waterdamp door de huid. Om een goed begrip van de daarmede samenhangende verschijnselen te verkrijgen is het noodzakelijk om op de hoogte te zijn van onderzoekingen, die aan synthetische en andere biologische membranen zijn verricht. Dergelijke onderzoekingen zijn in het eerste hoofdstuk ter sprake gebracht.

De grootte van het transport door een bepaalde membraan wordt, behalve door de concentratie- of drukgradiënt, bepaald door de soortelijke eigenschappen van het membraan, haar permeatie coëfficiënt. De invloed van de temperatuur, de absorptie van water (zwelling), en andere physische en chemische eigenschappen, die de permeatie beïnvloeden, worden in het eerste hoofdstuk besproken.

II Er is een kort overzicht van de beschikbare methoden gegeven, waarbij ook de argumenten worden genoemd, die aanleiding waren voor de ontwikkeling van een nieuwe meetmethode voor de bepaling van het verlies van waterdamp door de huid. Deze maakt gebruik van een warmtegeleidbaarheidscel als gevoelig element voor het aantonen van de hoeveelheden water. De keuze van een warmtegeleidbaarheidscel of katharometer berustte grotendeels op de wens om een methode te verkrijgen, waarbij de meting wordt verricht in de normale, vochtige lucht van de omgeving. Op deze wijze kan een meting sneller worden uitgevoerd, omdat de huid niet aan een andere atmosfeer behoeft te gewennen. Daarnaast kunnen invloeden van verandering van vochtgehalte van de atmosferische omgeving worden bestudeerd.

Er werd een warmtegeleidbaarheids-mikro-cel toegepast, die door de amerikaanse firma GOW MAC (vertegenwoordigende

firma in Nederland: Becker Delft NV) in de handel wordt gebracht. Het schema en de werkwijze voor de bepaling van het waterdampverlies door de huid zijn in het tweede hoofdstuk besproken. Het is gebleken, dat de gevoeligheid van de cel voor het aantonen van waterdampverschillen in de lucht zo groot is, dat meting op slechts één cm^2 onderarm-huid van de mens kwantitatief mogelijk is. De lucht wordt hierbij met een snelheid van 10 ml per minuut door een op de huid geplaatst kapje gevoerd. Hoewel men op grond van gegevens uit de literatuur anders zou kunnen verwachten, blijkt de gevoeligheid van het instrument gelijk bij elke vochtigheidsgraad van de lucht, zelfs voor droge lucht met slechts 1 % relatieve vochtigheid. Dit vergemakkelijkt de werkwijze voor de snelle bepaling.

Enige methoden ter ijking van de gevoeligheid van het apparaat, zowel direkte als indirekte methoden, werden toegepast. De gevoeligheid blijkt ongeveer 0,7 volt per gram water per liter lucht te bedragen. Er werd een nieuwe, eenvoudige, direkte methode ontwikkeld, omdat de bestaande methoden niet aan de gestelde eisen voor zeer kleine vochtigheidsveranderingen voldoen. In de praktijk kan de uitslag van het instrument kwantitatief worden vergeleken met die van een elektrolytische water analysator. Een dergelijke vergelijking werd tevens gebruikt om te controleren dat de uitslag van de warmtegeleidbaarheidscel bij meting aan de huid inderdaad betrekking heeft op het waterverlies door de huid en niet noemenswaard wordt beïnvloed door andere stoffen, zoals bijvoorbeeld kooldioxyde.

Een meting van het waterverlies door één cm^2 huid kan in normale vochtige omgevingslucht worden uitgevoerd in twee minuten.

III De plaats van de barrière van de huid tegen waterverlies is gelegen in de buitenste laag, de hoorn-laag of stratum corneum. Deze hoorn-laag, die ongeveer 0,02 mm dik is, kan laagsgewijze

worden verwijderd door hem weg te strippen met behulp van cellotape. Wanneer de gehele hoornlaag van de huid is afgestript, blijkt de resulterende weerstand van de huid tegen verlies van water tot op minder dan het honderdste deel te zijn afgenomen, en slechts 0,6 sec/cm te bedragen. Deze weerstand blijkt gelijk te zijn aan de weerstand tegen verlies van waterdamp uit een oplossing van albumine.

De weerstand van de waterbarrière van gezonde onderarmhuid voor verlies van waterdamp is ongeveer 300 tot 150 sec per cm. Ook voor vloeibaar water is deze weerstand zo groot. Men kan de huidbarrière tegen verlies van water dus gericht denken tegen het transport van watermolekulen, ongeacht de phase.

De weerstand tegen verdamping van water door een laag n-hexadecaan is gemeten met behulp van een elektrolytische water analysator, zoals ook voor meting van het waterverlies door de huid wordt gebruikt. Het n-hexadecaan kan als ijkstof worden gebruikt voor dergelijke metingen aan andere stoffen, die voor applikatie op de huid therapeutisch of voor andere doeleinden worden toegepast. Het n-hexadecaan is in de handel in zeer zuivere vorm verkrijgbaar en haar weerstand tegen watertransport is bekend. Op dezelfde wijze is ook de weerstand tegen verdamping van water door vaseline gemeten, omdat deze stof wordt toegepast wegens zijn occlusieve eigenschappen. Haar weerstand tegen verdamping bleek 600 sec/cm te bedragen voor een 0,01 mm dikke laag. Het afdekkend effekt van vaseline is vervolgens nagegaan door waterdampverlies metingen aan huid van de onderarm, voordat en nadat daarop vaseline was aangebracht. Uit de experimenten blijkt, mede door berekening van resultaten, dat de vaseline waarschijnlijk niet als een overal even dikke laag uniform op de huid aanwezig is. De faktoren, die de uitkomsten van deze experimenten kunnen beïnvloeden, zijn besproken.

IV Het verlies van water door de menselijke huid is geringer, wanneer het vochtgehalte van de omgevende lucht groter is. De relatie van beide faktoren volgt echter niet de wet voor de diffusie volgens FICK. De doordringbaarheid of permeabiliteit van de huid voor water is niet konstant en onafhankelijk van de vochtigheid van de lucht, maar is groter naarmate de atmosferische vochtigheid groter is. In dergelijke omstandigheden is het vochtgehalte van de hoornlaag groter en haar op deze wijze veranderde samenstelling kan de grotere permeabiliteit verklaren. Deze bleek in lucht van normale vochtigheidsgraad ongeveer 10 tot 20 % groter te zijn dan de permeabiliteit in droge lucht. Deze metingen werden mogelijk door toepassing van de warmtegeleidbaarheidscel.

Door de methode van meting met de warmtegeleidbaarheidscel is het ook mogelijk om na te gaan hoelang het duurt voordat het onmerkbare of insensibele waterverlies door de huid konstant is, nadat de zweetklieren door hun aktiviteit het huidoppervlak hebben bevochtigd. Dit bleek ongeveer een half uur te zijn. Een registratie van het waterverlies tijdens een proef na zweten is in het vierde hoofdstuk gegeven. Hierbij kon tevens worden berekend, dat het vochtgehalte van het stratum corneum na het zweten in dat half uur met ongeveer 4 % (absoluut) afneemt, dus in zekere zin ont-zwelt.

V De hoornlaag van de huid kan laagsgewijze worden verwijderd door hem zorgvuldig met cellotape te strippen. In welke mate daarbij het waterverlies door de huid wordt verhoogd, kan worden gemeten door het waterdampverlies van de gestripte huid te vergelijken met het waterdampverlies door naburige, normale en niet-gestripte huid. Door dergelijke metingen wordt een index voor de beschadiging van de waterbarrière van de huid verkregen.

De meting van de beschadigings-index van de waterbarrière van de huid kan tijdens de periode van herstel dagelijks

worden herhaald. Deze beschadigingsindex wordt als logarithme van het verhoogde waterverlies van de huid (IWVL) grafisch uitgezet tegen de tijd. Op deze wijze worden rechte lijnen verkregen. Uit de helling van de lijn kan worden afgeleid, welke de half-herstel-tijd van de huid is.

In figuur V.5 is een grafiek weergegeven, die als model dient voor het verloop van het herstel van de waterbarrière van de huid na beschadiging en is opgesteld aan de hand van metingen aan verschillende gezonde personen. Het grijze gebied geeft de verzamelplaats weer van toestanden van het waterverlies door de huid na beschadiging, wanneer daarbij juist het gehele stratum corneum en eventueel een gedeelte van het daaronder gelegen stratum granulosum was aangetast. Wanneer de huid verder is weggestript dan alleen het stratum corneum of ernstiger is beschadigd, wordt dikwijls een sterker verhoogd waterverlies door de huid gevonden, waarvan de huid zich echter zeer snel herstelt wat betreft de inperking van het waterverlies gedurende de eerstvolgende 4 à 5 dagen. Er wordt dan een zogenaamde "tijdelijke" barrière gevormd, die naderhand wordt afgestoten in de vorm van schilfers en parakeratotisch van aard is. In deze gevallen wordt daarna pas de definitieve barrière aangelegd, welke een normale herstelsnelheid blijkt te hebben.

VI Wanneer de huid aan alkali wordt blootgesteld, kan zij daardoor worden beschadigd, doch de huid gaat de beschadigende werking van de alkali tegen door haar te neutraliseren. De alkali-neutralisatie-proef (ANtest) en de alkali-resistentie-proef (ARtest), die al sinds vele jaren worden toegepast, worden beschreven en vergeleken met de recent uitgewerkte bepaling van de alkali-neutralisatie-snelheid (ANrate).

Wanneer de pH van de vloeistof lager is dan 10, blijkt experimenteel de alkalineutralisatiesnelheid gering te zijn en kwantitatief ongeveer even groot als kan worden verwacht wegens neu-

tralisatie van het kooldioxyde, dat door de huid naar buiten gaat. Bij deze pH blijkt de huid niet te worden aangetast door de loog.

Wanneer de pH van de vloeistof hoger is dan 11,5 en op die waarde gedurende een uur wordt gehouden, blijkt de hoornlaag van de huid te worden beschadigd. De beschadiging is duidelijk ernstiger, wanneer de pH slechts enkele tienden hoger is. De alkalineutralisatiesnelheid wordt nu ook veel hoger gevonden. Het verband blijkt uit de grafieken VI.4 en VI.2. De grens-pH, waarbij nog juist geen aantasting van de huid wordt verkregen, kan variëren en kan door applikatie van vaseline worden verschoven naar hogere waarden.

Wanneer de huid dagelijks regelmatig aan een alkalische vloeistof wordt blootgesteld, blijkt de huid daaraan te kunnen adapteren en niet zo ernstig te worden beschadigd als op grond van een eenmalige blootstelling verwacht zou worden. De mate waarin de huid respondeert aan herhaalde blootstelling aan alkali, is aan de hand van een voorbeeld weergegeven.

VII De huid werd eveneens gedurende zes dagen elke dag één uur blootgesteld aan oplosmiddelen (ethanol, ethylacetaat, methylethylketon en tolueen) of elke dag gedurende een kwartier aan sommige oplosmiddelen (chloroform, aceton, trichloorethyleen, dimethylsulfoxide en wasbenzine). De beschadiging, die hierdoor werd veroorzaakt, is nagegaan. Ook werd de herstelsnelheid van de huid na de beschadiging gemeten aan de hand van het verhoogde waterdampverlies van de huid. De huid blijkt dikwijls een zogenaamde "tijdelijke" barrière te vormen, die een goede bescherming biedt tegen volgende blootstelling aan het oplosmiddel. De beide laatstgenoemde oplosmiddelen, DMSO en wasbenzine, blijken een ernstiger beschadiging te geven onder de genoemde omstandigheden dan de overige onderzochte oplosmiddelen.

Industriëel kan de huid van de mens in kontakt komen met oplossingen van phenol in een resorcinol-formaldehyde hars. Een

2 % phenol oplossing in water geeft reeds een beschadiging, die vergelijkbaar is met die van 0,03 n NaOH. Een even ernstige beschadiging wordt bij een resorcinol-formaldehyde hars pas bereikt bij een concentratie van 13,5 % tot 18 % phenol. Blootstelling aan 18 % phenol oplossing in de hars bewerkstelligde de vorming van een meer effektieve "tijdelijke" barrière, dan bij blootstelling aan 13,5 % phenol oplossing in de hars het geval was. Wanneer de phenolconcentratie evenwel boven 18 % uitgaat, faalt de bescherming van de hoornlaag van de huid.

Het behoeft geen ernstige nadelige gevolgen te hebben, wanneer de hoornlaag van de huid zelf in eerste instantie in geringe mate wordt beschadigd, en deze beschadiging regelmatig wordt herhaald. De huid heeft dikwijls een effektief antwoord op een dergelijke reeks beschadigende invloeden, waardoor men in beroep en bedrijf geen ernstige klachten hoort. Desondanks kunnen beroepsomstandigheden soms kritisch zijn en kan een kleine overmaat (mechanisch zowel als chemisch) tot ernstige klachten aanleiding geven. In dat geval moet voldoende aandacht worden geschonken aan preventieve maatregelen, zodat de kritische drempel niet wordt overschreden. Enige van deze maatregelen worden genoemd.

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STELLINGEN

1: De konklusie van HERD & OPPERMANN (1966) "dasz der Diffusionswiderstand nicht von der Hauttemperatur beeinfluszt wird' gaat slechts in toevallige omstandigheden op.
E. HERD & Ch. OPPERMANN, Pflugers Arch. ges. Physiol. 291: 170 (1966)
D. SPRUIT, Am. Perf. Cosm. 81: 25 (1966)

2: Resultaten van lapjes-proeven met chromi-zout-oplossingen voor het aantonen van een allergische kontakt-overgevoeligheid voor chroom kunnen niet tot eenduidige konklusies leiden, tenzij de samenstelling van de verbindingen in de oplossing in redelijke mate bepaald is. Het verdient daarom aanbeveling om de chromizoutoplossing in onderdelen te scheiden over een kationenuitwisselaar, zoals bv. IMAC C 12 in de natriumvorm.
K.H. GUSTAVSON, J. Soc. Leather Trade's Chem. 35: 160 (1951)
J.W.H. MALI, K. MALTEN & F.C.J. van NEER, Arch. Derm. 93: 41 (1966)
J.E. WAHLBERG, Dermatologica (Basel) 137: 17 (1968)

3: De ontwikkeling van een allergische kontakt-overgevoeligheid is - althans voor sommige stoffen - een kwantitatief gebeuren, waarbij het vóórkomen van een drempel-concentratie waarschijnlijk is.
K.E. MALTEN & D. SPRUIT, Acta dermato-venereol. 49: 14-19 (1969)

4: Het is wenselijk om voor-nikkel-overgevoelige personen erop te wijzen, dat kontakt met nikkel als metaal of als legering zeer nauwgezet moet worden vermeden; dit is belangrijker dan vermijding van kontakt met sporen-nikkel-bevattende was-middelen of andere soortgelijke stoffen.
M.H. SAMITZ & H. POMERANTZ, A.M.A. Arch. ind. Hlth., 18: 473 (1958)
K.E. MALTEN, D. SPRUIT & W.Th.M. van HOORN-van GILS, Ned. T. Geneesk. 108: 1165 (1964)
K.E. MALTEN, K. SCHUTTER, K.G. van SENDEN & D. SPRUIT, Acta dermato-venereol. 49: 10 (1969)

5: De waarde van de verkregen informatie uit lapjesproeven op een aantal van ongeveer 100 tot 300 personen, die tevoren aan de te onderzoeken stof niet waren blootgesteld, wordt door de onderzoeker gemakkelijk overschat. De uitkomst van dergelijke proeven kan voor niet-ter-zake-deskundigen misleidend zijn.
Ch. HENDERSON & E.C. RILEY, J. invest. Derm. 6: 227 (1945)
A. ROSTENBERG, A.M.A. Arch. ind. Hlth. 20: 181 (1959)
Redactioneel artikel in Food cosm. Tox. 4: 525 (1966)

6: Toepassing van een "nieuwe" stof voor normaal gebruik in bijvoorbeeld voedingsmiddelen, kosmetika, e.d., dient na de gebruikelijke voorproeven en nadat de toelaatbaarheid en veiligheid voor gebruik waarschijnlijk zijn geworden, eerst anderhalf jaar op haar onschadelijkheid te worden gekontroleerd in een beperkte, doch zeer grote bevolkingsgroep (van bv. een miljoen mensen) voordat zij in de gehele wereld in de handel wordt gebracht.
C. PUGH, J. Soc. cosm. Chem. 18: 689 (1967)

7: Invoering van zogenaamde "positieve" lijsten ("permitted lists") voor voedingsmiddelen, kosmetika, e.d., kan alleen worden overwogen, wanneer men bereid en in staat is daarop tevens de "natuurlijke" voedingsmiddelen en grondstoffen te vermelden.
AANTEKENING BIJ redactioneel artikel betreffende: "Running neck and neck on croton oil", Food cosm. Tox. 4: 526 (1966)

8: Voor alle zalven en andere lokaal gebruikte therapeutika dient de basis (het vehiculum) te worden aangegeven.
I.H. BLANK, J. invest. Derm. 43: 415 (1964)
H.J. BANDMANN, Pharmaz. Z. 111: 1470 (1966)
N. HJORTH & K. THOMSEN, Brit. J. Derm. 80: 163 (1968)

9: Organische kwikverbindingen worden ten onrechte als niet-kontakt-sensibiliserend beschouwd.
S. FREGERT & N. HJORTH, Contact Dermatitis Newsletter, no. 5: 88 (Febr. 1969)

10: Het vóórkomen van formaldehyde (of formaldehyde-producerende stoffen, zoals hexamethyleentetramine) in als kosmetikum aangeboden artikelen dient te worden verboden.
R. BREIT, Contact Dermatitis Newsletter, no. 5: 94 (Febr. 1969)

11: Het is gewenst reeds nu de beweging langs de openbare weg in een stad te beperken tot die welke biologisch, elektrisch of nukleair wordt aangedreven: voor een stad, waarin een universiteit is gevestigd, is deze wenselijkheid zelfs urgent. Versnelde overgang van de traktie voor stedelijke vervoersdiensten op een elektrische is een afspiegeling van goede zorg van de stedelijke overheid voor het welzijn van haar stad en het behoud van de universiteitsvestiging binnen haar "muren".
1) Oliver Wendell HOLMES: "There is no form of lead-poisoning which more rapidly and thoroughly pervades the blood and bones and marrow than which reaches the young author through mental contact with type-metal." Troostend citaat; zie Food cosm. Tox. 4: 535 (1966)
2) T.J. HALEY: Chronic lead intoxication from environmental contamination: Myth or fact? Arch. environm. Hlth. 12: 781-785 (1966)

3) J.L. EVERETT, C.L. DAY & D. REYNOLDS: Comparative study of lead at selected sites in the British Isles in relation to air pollution. Food cosm. Tox. 5: 29-35 (1967)
4) V.J. KONOPINSKI & J.B. UPHAM: Commuter exposure to atmospheric lead. Arch. environm. Hlth. 14: 589-593 (1967)
5) R.O. BECKER, J.A. SPADARO & E.W. BERG: The trace elements of human bone. J. Bone Joint Surg. 50 A: 326-334 (1968)
6) P.V. BASCH: Contribution à l'étude de l'oxycarbonisme chronique dans un milieu industriel. Diss. Strasbourg (1960)
7) S. Åke LINDGREN: A study of the effect of protracted occupational exposure to carbon monoxide, with special reference to the occurrence of so-called chronic carbon monoxide poisoning. Acta med. Scand., Suppl. 356, 1-135 (1960/61)
8) R. TRUHAUT, C. BOUDENE & J.-R. CLAUDE: Fixation thyroidienne de l'iode131 chez le rat intoxiqué chroniquement par l'oxyde de carbone. Anns. Biol. clin. 23: 73-82 (1965)
9)R.J.M. de JEEUW, M.L. HAMELINK, J. KREUKNIET, B.F. VISSER, J.C.M. DOUZE & A.N.P. van HEIJST: Koolmonoxidebepalingen in bloed. Commentaar . . . .
Chem. Weekblad 62: 591-592 (1966)
10) H. BOUR, M. TUTIN & P. PASQUIER: The central nervous system and carbon monoxide poisoning. I. Clinical data with reference to 20 fatal cases. in: H. BOUR & I.McA. LEDINGHAM: Progress in Brain Research: Vol. 24, Carbon Monoxide Poisoning; Amsterdam, Elsevier, pp. 1-30 (1967)

12: De keeper en de scheidsrechter wordt alleen hun feilen aangerekend; de spelers in de voorhoede alleen de ballen, die doel treffen. Het is een edukatieve taak om te onderwijzen, dat in àlle gevallen het gehele elftal de basis legde èn aansprakelijk is.
Hetzelfde geldt voor de gemeenschap van wetenschappelijke werkers in het onderzoek.